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CONTENTS

	PAGE
Healing of End-to-End Intestinal Anastomoses With Especial Reference to the Regeneration of Blood-Vessels. (Illustrated.) By Florence R. Sabin	289
End-to-End Anastomosis of the Intestine by Presection Sutures. An Experimental Study. (Illustrated.) By Emile Holman	300
An Instance of Voluntary Acceleration of the Pulse. (Illustrated.) By John T. King, Jr.	303
The Innervation of the Tensor Veli Palatini and Levator Veli Palatini Muscles. By Arnold Rice Rich	305
Anaphylaxis. (Illustrated.) By H. H. Dale	310
The Natural History of Typhoid Fever in Baltimore, 1851-1919. (Illustrated.) By William Travis Howard, Jr.	319

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HEALING OF END-TO-END INTESTINAL ANASTOMOSES WITH ESPECIAL REFERENCE TO THE REGENERATION OF BLOOD-VESSELS

By Florence R. Sabin

(From The Department of Anatomy, Johns Hopkins Medical School)

In the spring of 1919 Prof. W. S. Halsted returned to the study of methods of end-to-end anastomoses of the intestine, a subject on which he had worked with Dr. Mall in 1887. At that time Dr. Halsted originated the operation while Dr. Mall had shown that histological studies were not only essential for understanding the processes of repair but could also be made an aid in the developing of surgical technique.

In relation to these studies, Dr. Halsted suggested to the writer that the application of some of the improved methods in connection with vascular injections, as applied to the healing of blood-vessels in these anastomoses, might throw some light on the general problems involved in the healing of intestinal wounds. Having had the privilege of working for many years under the stimulus and guidance of Dr. Mall, and having long known of the pleasure he had taken in this work with Dr. Halsted, it was to me a great gratification to have a share in the study of their problem.

After the first two operations, which were performed by Dr. Halsted, the surgical work was done by Dr. Emile F. Holman, whose analysis of the surgical results will be found in the same number of this journal.

As in the original experiments of Halsted and Mall, the animals used were dogs. The series studied by me comprised 25 dogs, of which 20 were injected, involving 27 anastomoses. Sections were cut from all the injected specimens and from three of the uninjected. All of the anastomoses but one were made in the small intestine, that one being in the large intestine; five were in the duodenum and the rest in the upper part of the jejunum. The animals were killed and the injections made at varying intervals after the operation; in one case the injection was made immediately after the operation; the other animals were injected after intervals of from 1 to 69 days.

The injections were made with India ink, diluted one half and made alkaline with a few drops of ammonia. The cannula was inserted into the superior mesenteric artery and a vent was made in the portal vein. After the blood had been washed out with normal salt solution the ink was introduced and injected at a pressure from 100 to 110 mm. of mercury until the intestine looked quite black, when the opening in the portal vein was clamped for a few seconds to insure filling the capillaries. The entire small intestine was then removed

with all the vessels tied off and placed in 10 per cent formalin for 24 hours, after which the areas of the anastomoses were placed in 80 per cent alcohol. After 2 or 3 days in alcohol longitudinal strips were cut through the anastomoses and cleared by the Spalteholz method, while adjacent strips were embedded in celloidin and cut at varying thickness, from 10 to 100μ, and stained in hæmatoxylin and eosin or erythrosin, in alum cochineal, Mallory's connective-tissue stain, and in toluidin blue. The blocks, cleared by the Spalteholz method, were thoroughly dehydrated by running through graded alcohols, using two changes of absolute alcohol; they were then passed through benzine into oil of wintergreen. If when cleared these specimens are too thick to be readily analyzed, they can be cut thinner with a sharp Gillette blade.

In his histological studies Mall divided the healing of intestinal wounds into four stages: (1) The immediate union of the serous surfaces by fibrin; (2) the destruction of redundant tissue in the flaps; (3) the regeneration of the mucosa and (4) the straightening of the intestine. Both Halsted and Mall found that the union by fibrin could be noted even before the operation was completed, and this proved to be of great importance in eliminating what Mall called a "dead space," for when this space formed, it always filled in with leucocytes. There were two factors in its prevention, first the fibrin, and second, the firmness of the stitches, which Halsted showed must enter the submucosa in order to hold securely.

Mall followed the regeneration of the mucosa at the cut edges which took place during the first three weeks. The results depended largely on the accurate apposition of the different coats, submucosa to submucosa and mucosa to mucosa. He noted the retraction of the muscularis mucosæ and stratum fibrosum at the tip, leaving the submucosa exposed and showed how the epithelium regenerated over this area, soon making glands of the simple embryonic type and then villi. He noted that these newly formed glands dipped down into the submucosa and described the new blood-vessels of the mucosa as simulating the pattern of the vessels of the stomach or at least that of the zone of transition between the stomach and duodenum. Perhaps one of his most interesting points was the showing how the intestine rights itself—that is, how the infolded ends straighten out. In the end-to-end anastomosis, most of the infolded parts are viable and the intestine straightens out in the following manner; the inner sutures pull out and are discarded into the lumen of the bowel, while the outer row of sutures pull out of the submucosa into the muscularis, then through the muscle into the peritoneal cavity. In this process, the silk being tied, is anchored either at a point exactly opposite the site of anastomosis, as in Mall's Fig. 12, or may pull out far to the side as in my Fig. 19. The straightening takes place during the fourth week, when the regeneration of the muscularis mucosæ and the stratum fibrosum takes place.

In following the series which I have studied, the most striking point about the form of end-to-end anastomosis, as it has been developed by Dr. Halsted and Dr. Holman, is the very slight damage to all of the tissues and therefore the slight amount of regeneration called for. This is of course bound up with the fact that there is practically no interference with the blood supply. This is shown in Fig. 1, which is from an anastomosis in the jejunum taken 21 hours after the operation. Here, as will be seen in the photograph, the apposition is perfect, the infolded parts of the intestine being held firmly by fibrin, since there is no suture at this place. The fibrin shows as a dark streak in the photograph with a triangular mass in the serosal border, and it has practically no infiltration with leucocytes. In no other case in our series has the union by fibrin been as firm as this, for though in all but one of our specimens of the first four days the union was firm enough for physiological purposes, still, in cutting out the blocks it was difficult to draw the knife through the anastomoses without loosening the two infolded parts. It will be noted in Fig. 1 that the mucosa is practically intact, with normal villi except at the tip of the anastomosis where the cut was made, and for a short distance on either side of the apex. In this connection it must be noted again that the method of fixation, depending on the passage of formalin through the entire wall of the gut instead of being injected into the vessels or even poured into the lumen, makes it impossible to avoid some damage to the villi due to post-mortem changes. However, a specimen such as this would call for virtually no destruction of redundant parts and only the tip of the anastomosis would need a regeneration of the mucosa to cover the exposed submucosa. In his studies of gastro-enterostomies in dogs, Flint described a sloughing of the mucosa subsequent to the operation, due to a local cutting off of the blood supply. Dr. Holman's form of suture, running as it does parallel to the largest vessels of the wall, *i. e.*, to the vessels of the fourth order, seem to me to be especially adapted to avoid interference with the blood supply. In the specimen shown in Fig. 1 it is true that the injection mass does not fill the vessels of the villi, but the same is true of this entire intestine and means simply that the injection was not pushed far enough to be complete. This has happened in one or two of our experiments. On the other hand, in Fig. 16 is seen a practically complete injection, with the villi intact to the edge of the suture. This is from a case injected 4 days after operation. The section is cut thick in order to show a wide serosal margin in connection with the new growth of vessels; it has no extravasations and represents very well the average of the injections of early stages and so shows that this form of anastomosis need not produce any localized interference with the blood supply.

In testing out the question as to when vessels cross from one of the infolded flaps to the other, we made some experiments of tying off the vessels of the mesentery of one side and were surprised at the amount of filling of the vessels along the wall of the intestine; thus the tying of one of the radiating vessels of the mesentery, and one vascular loop does not prevent the filling of the vessels of the corresponding wall of the intestine. Whether or not it is possible to make as complete a preservation of the blood-supply, both of the stomach and of the intestine, in a gastro-enterostomy can be very

readily tested by similar experiments with injected specimens, but I am confident that these studies prove the matter in the case of the intestine. Flint indeed pointed out the very close relation between the rapidity of the processes of repair and the amount of damage to the blood supply.

In one of our dogs, the nineteenth in the series, three anastomoses were made. The first was very successful and is shown in Fig. 6. Fifteen days after this operation two more anastomoses were made and the animal was killed the next day. In the case of the third anastomosis, a different operation was done; two presection, stay sutures were laid, then the bowel was cut and sutured with two rows of through and through stitches. At the operation Dr. Holman had difficulty in burying the mucosa, so he re-inforced with five Halsted mattress sutures and several Lembert stitches. During the process the wall of the bowel became blue and at the time of operation Dr. Holman noted the method as unsatisfactory. Histological studies confirmed this judgment. The lumen of the gut contained much blood and sections show a considerable mass of clotted blood adhering to the area of the anastomosis. The villi over the anastomosis showed marked capillary hæmorrhages, being, in fact, completely filled with corpuscles. The ink extravasated in the submucosa and around the crypts and did not enter the villi at all. In this case, therefore, there was extensive damage to the walls of the blood-vessels in the infolded area. It is interesting to note, however, that in spite of the unsatisfactory nature of the last operation and the fact that it was the second time the abdominal cavity was opened, there were no signs of a beginning peritonitis when the dog was killed. Another operation done on the thirteenth dog, with Connell mattress stitches around one half of the lumen and an over-and-over continuous stitch for the rest with reinforcing sutures, was also proved to be unsatisfactory by the sections, as they showed considerable damage to the mucosa and a very marked infiltration of the entire infolded musculature with leucocytes.

In the study of the early cases, that is, in those of four days and under, of which there were ten, there were signs that the mucosa was not only intact but that the villi might function. In other words, changes in the epithelium over the villi, which Mingazini has recognized as evidences of the process of the absorption of substances other than fat, were present. Any judgment as to whether these changes are physiological or post-mortem, however, is so dependent on perfect fixation that no stress can be laid on them with a fixation such as one gets in injected specimens, where the lumen is tied off to prevent leakage of the injected mass. Consequently, the following experiment was tried: Dr. Holman made an end-to-end anastomosis of the jejunum of another dog, and five days later a second anastomosis of the jejunum was made. The dog was not fed at all until the second day after the last operation, when he ate an enormous meal of meat, potato and cream. Five and a quarter hours later he was killed and the typical picture of fat absorption was found. The lymphatics of the mesentery, as well as the thoracic duct, were full of chyle. The superior mesenteric artery was injected with 10 per cent formalin to fix the chyle in place; frozen sections were then made through each anastomosis and through other parts of the jejunum and stained with Sudan III. The villi over both anastomoses were shown to be as active in the absorption of fat as was the rest of the wall by the presence of fat in the epithelial cells, in the central lacteals of the villi as well as in the lymphatic vessels of the submucosa. Moreover, the villi over the anastomosis which had been made two days before were as active as those over the anastomosis which had been made seven days previously. Thus there was no interference in the function of the villi in the absorption of fat, nor was there any dilatation of the lymphatics in the submucosa which would have followed had there been an interference with the drainage of the absorbed material away from the wall. The lymphatics in the mesentary opposite the areas of the anastomoses could not be seen owing to an extensive adherence of the omentum which took place after the second operation. A block from the second operation was then embedded in celloidin and the sections showed the typical figures of absorption indicated by Mingazini. The epithelial cells are raised from the stroma, the inner borders of the cells being stretched out but not yet ruptured, except at the tips of the villi, while throughout the stroma of the villi are very many plasma cell. Thus the proof that the operation does not inhibit the function of the villi in absorption is complete. From these studies I think that practically the entire infolded ridge is viable and can function so that there is little tissue that is redundant, and hence exceedingly little early destruction.

In contrast to this is the section in Fig. 3 from a case of an anastomosis in which a Murphy button had been employed. In this case the animal was injected immediately after the operation. This section is from the end of the bowel which was the more damaged and shows four zones, one in which there is destruction of all of the mucosa; second, a zone in which only the bases of the crypts are left; third, an area in which all of the villi are gone, and fourth, the intact mucosa. A part of the damaged wall was inclosed in the button and hence would have sloughed off but there was a considerable area of destroyed mucosa outside of the button, showing that the operation produces an extensive ulcer beside the crushed wall which must slough off with the button. The injection is strikingly poor around this end of the anastomosis, though excellent in the other end where the muscle is more strikingly damaged. The well injected end shows marked extravasations of the injected mass, which follows of necessity from the amount of destruction. In our one case there was no chance to follow the processes of repair, since the dog was killed immediately after the operation; but sections show a great amount of damage to the intestinal wall, for there are hæmorrhages in some places, and in others a blocking of the circulation and an extensive recent ulcer which must be repaired.

In following the healing of the series of anastomoses, I shall consider three things: (1) the healing of the mucosa, (2) the

amount of damage to the smooth muscle, and (3) the regeneration of the blood-vessels.

Mucosa

The processes in the regeneration of the mucosa can be followed in a series of photographs in Figs. 1 to 12, representing stages of from 1 to 69 days. As has been seen in Fig. 1, if there is a perfect apposition of the two parts there is very little need for the regeneration of the mucosa. However, it is hardly possible to have the apposition quite as perfect at the cut edge all the way around the lumen of the gut. There is, of course, the well known tendency of the mucosa to roll outward at the cut end, and while tying the last two of the second row of stitches the mucosa must often be rolled in under the sutures and one cannot see the exact apposition. In 4 days, unless there has been an exceptionally wide zone of the submucosa exposed, the entire gap of the submucosa is covered by a layer of simple, cubical epithelium. Flint found that this epithelial covering began to spread across the gap as early as the first day. By the seventh, certainly by the ninth day, as shown in Fig. 4, the gap in the mucosa is bridged by simple glands of the embryonic type as described by Mall, that is to say, with glands with high columnar epithelium and many nuclei, often several rows deep and without any differentiation of the cells.

After the glands have formed, a few short, broad villi begin to appear, as shown in Fig. 5, from an anastomosis 14 days after the operation. In this figure is one conspicuous new villus exactly opposite the very apex of the anastomosis. In this case the apposition has been very perfect. The section is through one of the basal stitches around which is a slight localized reaction of leucocytes and giant cells of the type associated with foreign bodies, but there is not the slightest trace of an inflammatory reaction along the serosal margin of the interned flaps.

At the tip of the anastomosis the characteristic retraction of the muscularis mucosæ is very plain. The newly formed glands, of an embryonic type, have grown down into the submucosa between the cut ends of the muscularis mucosæ. The glands are, however, not as simple as at first, for there is now a beginning of a differentiation of mucous cells in them. The differentiation of an excessive number of mucous cells in the crypts of Lieberkühn over the infolded area is a constant phenomenon from this time on. It shows especially well in Figs. 11 and 12, 50 and 69 days after the operation, in which the pale areas in the crypts are due to an excessive number of mucous cells. In many cases the crypts come to look like glands of the large intestine. For example, in Fig. 13 we have a photograph of a small area from the mucosa of Fig. 7 in which every cell of the glands in a certain zone is a mucous cell.

Fig. 11 is interesting as showing the fate of these areas of overgrowth of mucous cells. It will be seen in the photograph that there are alternating dark and light areas in the crypts; the light areas are masses of mucous cells showing much vacuolization and marked signs of degeneration, the part of the cytoplasm next the lumen being almost destroyed, while the dark areas represent zones of young cells with a striking number of mitotic figures. Over these areas there was much mucus in the lumen of the intestine. In Fig. 12 is shown a very extensive area of mucous cells in the crypts covering nearly the entire infolded flap and the wall to the right almost to the edge of the section. It seems that the mucosa functions normally at the start and then differentiates a very great excess of cells secreting mucus; these eventually degenerate and are replaced by new cells. This condition seems to be constant, at least it is present in all of our cases above two weeks. It is interesting to note that this destruction of tissue is not directly due to the operation, rather, it seems to me that the infolded ridges are viable, but there is a late destruction of mucous cells which have developed in excess of the normal number as a result of the operation, and are subsequently destroyed and replaced by new cells as a functional adaptation.

Another point of great interest in connection with the mucosa comes out in Fig. 5, that is, the relation of the glands of the mucosa to the muscularis mucosæ. Wherever the muscularis mucosæ is broken the glands grow down into the submucosa, provided the area is not infected. At the cut edges, as seen in Fig. 5, the muscularis mucosæ is markedly retracted. Immediately after the operation, that is in twenty-four hours, the glands sag into the gap, after which they actively grow down by cell-division. This downgrowth of the glands is interesting in connection with the theory that Brunner's glands develop before the muscularis mucosæ forms. Elsewhere the muscularis mucosæ and stratum fibrosum seem to make a barrier for the glands.

There are three places where this downgrowth of the glands occurs; (1) at the tip of the anastomosis, as shown in Fig. 5; (2) opposite the outer row of stitches, as seen in Figs. 4 and 6; and (3) opposite the basal row, as seen in Fig. 8. In all of the cases in which the growth was opposite the sutures I feel sure that the muscularis mucosæ was simply ruptured by the pull of the stitches; indeed in one case the muscularis has been pulled to a sharp angle and not broken. When the stitch actually pierces the mucosa, an abscess forms, as shown clearly in Fig. 12, where the right hand flap is still infolded, while the left is entirely straight. The position of the inner left suture, which has been cast off into the lumen of the bowel, is indicated by the cavity of an abscess now well walled off, and here there has been no new growth of glands whatever.

In Fig. 6 there is a small cyst at the base of the little group of glands that have grown into the submucosa. The formation of these dilatations is characteristic and was noted by Mall. In the same figure there is a break in the mucosa, associated with the process of the casting off of the inner stitch into the lumen of the gut. Indeed, there are some strands of the silk already within the lumen. This is a case 16 days after the operation. Around the suture there is a reaction characteristic of foreign bodies, consisting mainly of clasmatocytes, which are the cells of the connective tissue, identified by Maximow as a special phagocytic group, resting wandering cells, which here become active, together with a few giant cells,

and a few leucocytes. I judge that this reaction, which is not of the acute inflammatory type, has been enough to spread the two muscle coats apart. There is one more interesting point about this reaction, namely, that the cavity in which the stitch lies is lined with simple epithelium. It is not possible to say whether this downgrowth took place immediately after the operation, due to an imperfect union by fibrin, or whether it is secondary to the process of the casting out of the suture. The cells do not look like new cells and in one case, shown in Fig. 10, there is a similar cavity lined with epithelium around one of the basal stitches which must have formed just after the operation. This simple epithelium, lining the cavities of the sutures, develops mucus cells. It seems to me such cysts must be abnormal and they make it worth while to stress the importance of firm apposition of the infolded parts. For instance, a slipping of one of the first line of sutures would bring about such a result, so that the first row should be tied with exactly the same care as the second row. A space like the one in Fig. 6 would probably be completely obliterated in the healing following the casting off of the stitch into the lumen of the bowel; but in the case of a basal stitch like the one in Fig. 10, which will ultimately work its way into the peritoneal cavity, the fate of such a cyst is by no means so clear and therefore must be regarded as a disadvantage.

The sections shown in Figs. 8 and 9 are from the same anastomosis, in fact they are cut from the same block. In Fig. 8 is shown a downgrowth of the glands opposite one of the basal stitches on the left side of the photograph. Just at the left border of the cyst around the suture is a little epithelium, evidently derived from the aberrant glands and not by a downgrowth from the apex of the anastomosis. The other section shows another very interesting result of the rupture of the muscularis, that is, the formation of an extensive compound lymph gland. This lymph node lies opposite a basal suture which was on the right side of the section and has been cast off. The node is exactly like a Peyer's patch, with the same breaking of the muscularis mucosæ and the same rather simple glands above it, although this section is from the duodenum. In this instance I assume that the muscularis mucosæ was ruptured opposite a small lymph follicle and that the injury stimulated the growth of the follicle instead of the glands of the intestine. In fact, the glands are less developed than usual, for in the other section, (Fig. 8) the right border of the infolded area has a completely regenerated, simple epithelium, with almost no glands. This area is on the edge of the lymph node; in fact the dark spot in the center of the area is an injection of lymphoid tissue. The thinness of the epithelium here, in so late a stage (25 days after the operation) may be associated with one of three conditions; viz., the development of the lymphoid tissue may have inhibited the glands, there may have been a secondary break in the mucosa associated with the casting out of a stitch, or there may have been an area of sloughing of the mucosa, such as described by Mall and Flint. I think that the first condition is the most probable one.

In following the repair of the intestine, it is a very interesting point to know the fate of these downgrowths of glands into the submucosa. The constancy of the reaction when the muscularis mucosæ is ruptured without infection indicates that the muscularis is a normal barrier to the excessive growth of the glands. In one of Mall's cases (see his Fig. 11) the growth of the glands was so extensive that he termed them an adenoma. But the reason that such a disturbed balance between two tissues has not led to the formation of adenomata in clinical experience is that the muscularis mucosæ grows down around these new glands and restores them to their normal position in the mucosa.

This is brought out in Fig. 10 from a case 38 days after operation, in which there has been a very deep downgrowth of the glands at the cut edge. This case would have shown a perfect result but for the fact already brought out that the epithelium has grown down to one basal stitch and formed a cyst around it. It is especially interesting as showing the formation of a new muscularis mucosæ. From both cut edges of the old muscularis mucosæ, as well as from the edges of the old circular muscle, bands of muscle are growing to form a new muscularis mucosæ, as is shown at a higher magnification in Fig. 14. In this figure the muscularis mucosæ on the lower margin of the photograph has nearly been completed from the old muscularis mucosæ and from a strand from the circular muscle. Around the base of the glands, shown at the right of the photograph the muscularis mucosæ has been completed from the circular muscle, while the upper border of the mucosa has many bands of smooth muscle cut in cross-section. These accessions of muscle from the old circular muscle coat were seen by Flint in his study of gastro-intestinal sutures.

When the regeneration of the muscularis mucosæ is complete and the anastomosis has straightened entirely, such a small villus as the one shown at the depth of the cleft in Fig. 14 will be restored to its normal position. I have not followed the complete regeneration of the muscularis mucosæ, that is, in all its layers. As is well known, this structure is elaborate in the dog, having an outer longitudinal layer and an inner circular layer with the stratum fibrosum. Besides these layers, longitudinal bands of muscle run out into the villi, so that the villi must have a three-way motion in the lumen of the intestine. In the regeneration I have no stages showing the complete restoration of this elaborate pattern, but there is a new simple muscularis mucosæ in Figs. 11 and 12, 50 and 69 days after the operation. Indeed the tendency to the completion of a muscularis mucosæ is constant and hence it must have an important function. I have tried to test the question whether all of the new muscularis mucosæ comes from preceding smooth muscle or whether there is a differentiation of new muscle cells in the intestine, without being able to solve the question with this material. Certainly almost all of the new muscle I find is connected with the old, and the small masses of isolated muscle along the upper border of the glands in Fig. 14 might well prove in serial sections to be connected with the old musculature. It is hard to

believe, however, that this can be true for the few muscle cells which are found in such a new, small villus as that seen in the same figure.

The regeneration of the muscularis mucosæ begins early; in one case studied 9 days after the operation there were strands of muscle streaming out from the cut end of the muscularis mucosæ at the apex of the anastomosis. It is well under way at 38 days and complete at 50 and 69 days. It is thus restored much more quickly and indeed far more completely than the main muscle coats.

Smooth Muscle

At the beginning of the work I expected to find the most marked signs of injury in the wall of the intestine in the mucosa, and this apprehension was due to the care which the histologist has learned to take in the fixation of intestine in order to secure an intact mucosa. To my surprise, however, the mucosa was strikingly intact, but on the other hand, there were distinct signs of damage to the musculature of the wall and these signs showed in the cases as early as 24 hours after the operation. For example, on the left side of Fig. 1, just at the turn in the muscular coat, there is a shadow. This is due to a deposit of fibrin and to an irregularity in the staining reaction of the muscle. This particular damage, I think, was due to the placing of the clamp by which the intestinal contents were kept from the operative field. The clamp was covered with rubber tubing and placed carefully, but the muscle certainly shows signs of injury.

In a subsequent case, (Dog 25) Dr. Holman placed the clamps 3 to 4 inches from the operative field and adjusted them with the greatest possible care. Sections were then cut through the zone of the clamps and showed slight mottling of the cytoplasm of the muscle, so that I think one may say that it is not possible to place clamps, even covered with rubber, with such care that signs of injury to the muscle may not be detected histologically. This would certainly make it worth while to stress the danger of rough handling of tissues containing smooth muscle.

Fig. 15 shows another striking instance of damage to the muscle. It is from the infolded musculature in a dog killed 24 hours after operation. Otherwise, this case showed a beautiful result with no abnormality except a slight infiltration of leucocytes. The light streak at the upper right corner is a space between the infolded serosal coats, perhaps produced by the knife. The dark band to the left is the much damaged longitudinal coats which have reacted with great intensity to the stain, while the circular muscle occupying the main part of the section is much mottled, the dark transverse band containing nuclei that are all pyknotic. All of the early cases show some damage to the muscle, due in part to clamps, but in part also simply to handling, as in this specimen. The case from which Fig. 15 is taken (Dog 12) is an instructive one. An anastomosis was made with practically perfect results, and 24 days later two anastomoses were made at the same time. The first, shown in Fig 15, was of the presection type, while the second was with sutures of the through and through type. The animal was killed in 24 hours and it is very striking that the second operation, that is, the one with through and through sutures, shows very much more inflammatory reaction than the first, giving a good chance to contrast the type of suture that passes through all of the coats with the Halsted type that simply enters the submucosa. The case from which Fig. 15 is taken showed excellent apposition; the wall injected very completely and the only defect was the damage to the muscle, which, being infolded and therefore thrown out of function by position, probably would not do serious harm. This case also brought out another point of interest; at the time the dog was killed there was no peritonitis, though it was the second time the abdominal cavity had been opened. In some other cases when the second operation was made very soon after the first it was followed by adhesions. This is, of course, a common surgical experience. To sum up our results in this regard, in the case of Dog 3 there was a very excellent local result of the anastomosis, as shown in Fig. 7; then after 14 days a second operation was done and the animal killed 8 days later. Following the second operation there was a matting of the loops of the bowel with a firm adhesion of the omentum to the second anastomosis. It was later proved that one suture had slipped, thus fully accounting for the poor result. It was interesting in this case that no difficulty was suspected before the animal was killed. On Dog 25, a second operation was done five days after the first operation and when the animal was killed, two days after the second operation, there was an extensive adhesion of the omentum to the bowel, including both anastomoses and the entire surface of the intervening mesentery which had been handled. Since in this case both anastomoses were excellent (indeed this was the case in which we tested the function of the villi after the dog had eaten an enormous meal), the adhesions probably represented the difficulty, well known to surgeons, of handling the peritoneum before it has entirely recovered from the effects of a first operation. The wall of the bowel was in good condition in this case but the omentum itself was really infected. A third case (Dog 16) was injected three days after an anastomosis had been made, and this animal showed an acute peritonitis. This was the only case with an infection following one operation, and it was especially instructive in showing a very extensive damage to the musculature around the anastomosis. In such a case the damage to the muscle is readily accounted for, but in almost all of our other cases there was very little inflammatory reaction; for example, in Fig. 22, it will be noted that the muscle shows no infiltration of leucocytes at all, the section being taken from a dog which was killed four days after operation. In such cases it becomes interesting to know whether the injury to the muscle is due to direct handling or to damage to the sympathetic cells; therefore, I had sections stained in toluidin blue but could find no marked changes in the ganglion cells. The nuclei are often excentrically placed, even at the edge of the cell, but there is no disappearance of the Nissl substance. Of course an occasional ganglion occurring near a stitch is entirely destroyed, the nerve cells being re-

placed by connective tissue, but for the most part Auerbach's and Meissner's plexuses do not seem badly injured and I therefore conclude that the striking histological changes in the muscle, as seen in sections, are due to the direct effect on the muscle itself.

The regeneration of the muscle coats is bound up with the process of the pulling out of the stitches and the straightening of the intestine and belongs therefore to a later period, namely the fourth week. The process of straightening of the intestine is an interesting one. In this regard, that is to say, in connection with the ultimate result of the restoration of the intestine to its normal condition, our studies, as far as they go, show a very decided advantage of the end-to-end anastomosis over the end-to-side form. In the one case of the latter, as Dr. Holman has described, there is a decided dilatation of bowel and an obvious stasis of materials in the dilated pouch, while in none of the end-to-end anastomoses was there such a dilatation nor any stasis. Even where the infolded flaps are fairly extensive, as in Fig. 5, there was no sign whatever of any blocking of the lumen.

The straightening out of the wall depends on the fate of the basal stitches, Figs. 7, 8, and 9 representing the most completely straightened wall in our series. In each case one side has straightened out more completely, while the other side is slightly raised by a thickened or possibly by a contracted musculature. The two oldest cases (Figs. 11 and 12) happen to show a less straightened wall. In both the suture has pulled out of the submucosa, through the muscularis, into a thickened serosa and is still holding there with very little cellular reaction. In the one case the suture holds both flaps symmetrically, whereas in the other it holds only one. In the case shown in Fig. 11 the opposite side of the bowel in the gross specimen is almost straight, hence the folding shown in the photograph is a local reaction to one stitch. In the injected specimen shown in Fig. 19, on the other hand, the basal suture has been pulled to the side of the anastomosis, so that the actual site of the anastomosis, which is opposite the depression in the mucosa at the extreme left, is thinner than the adjacent wall. It would seem, therefore, that the position of the sutures, determined perhaps by their firmness when there is little inflammatory reaction, controls the time of the final straightening; but in any event, an infolded wall seems to be no disadvantage, whereas a dilated wall is a distinct abnormality.

As can be seen in most of the later figures, there are ultimate gaps in the musculature, after the sutures have pulled through, that are not regenerated, certainly not during the interval we have studied. The fact that these gaps are localized opposite the six stitches around the lumen of the gut, so that the musculature is not completely isolated around the wall, probably makes them of less functional significance. Certainly, the lack of dilatation of the wall in all of our cases proves that the muscle is not sufficiently weakened by this type of operation to cause any thinning out of the musculature.

Regeneration of the Blood-Vessels

The regeneration of the blood-vessels in these anastomoses is one of the most interesting parts of the subject, both in relation to the understanding of the processes of repair in adult tissues and in relation to recent work on the embryological aspects of the vascular system. The development of methods by which it is possible to keep the living blastoderm of a chick under observation during the second day of incubation has made it possible to find out just how the vascular system begins, that is to say, to unravel its fundamental morphology. The blastoderms were first grown on a coverslip in a hanging drop preparation by McWhorter and Whipple who followed Harrison's technique of using clotted plasma. An easier technique, and I think a more valuable one for this particular purpose, is that of Lewis and Lewis, in which the blastoderms are grown in Locke-Lewis solution, which is Locke's solution to which 20 per cent of chicken bouillon has been added.

By this method every stage of the origin of blood-vessels can be seen with great clearness in the area pellucida (Sabin, 1920). Blood-vessels begin by the differentiation of a new type of cell, the angioblast, from mesoderm. Masses of angioblasts are of course the well known "blood-islands" of the early embryologists. This process begins in the embryonic membranes; in the chick, first in the area opaca. After such masses have differentiated in the area opaca they differentiate in the area pellucida, and then in turn in the embryo itself so that there is a progressive differentiation of angioblasts from without inward.

Angioblasts have certain characteristics. Their cytoplasm is denser and more basophilic than mesenchyme and, after fixation in Helly's fluid and staining with azur-eosin, they show the well-known basophilic granules depicted in Maximow's and Danchakoff's figures. These characteristics are, however, not wholly adequate for identifying a single angioblast; but after the first division angioblasts can be distinguished by their tendency to form characteristic clumps, apparently syncytial masses. Mesenchyme cells separate after division, but the angioblasts stay together and soon send out characteristic sprouts by which they join similar masses and form plexuses. These plexuses of solid cells are the forerunners of blood-vessels. The lumen of the vessel forms by the liquefaction of the central part of the cytoplasm to make blood-plasma and this liquefaction may take place in a given mass of angioblasts either before or after it has become a part of a plexus. If it takes place while the clump of cells is isolated, a vesicle is formed. The liquefaction of the cytoplasm has been seen in chains of single angioblasts as well as in the clumps, and hence it is actually an intracellular process; the edges of the masses remain as endothelium.

This method of the formation of blood-vessels is fundamental in the chick; the endocardium of the heart and the entire dorsal aorta behind the head fold can be seen to differentiate *in situ* in the living form by this process; moreover, not only the heart and the aorta but the primary vessels of the embryo

are formed in the same way. It is of course true that both angioblasts and their descendants, the endothelial cells, once differentiated, keep on dividing, so that the primitive vascular system is increased by the differentiation of new cells and the division of the old. It therefore becomes of great importance to know when and where the process of differentiation of new cells to make endothelium ceases. It may then be said that the fundamental morphology of the vascular system has been established and rests on a firm basis as a foundation for the study of vascular problems. On this foundation, it is important to know, even if the normal repair of vessels be from old endothelium, whether there is a reserve, in the adult body in general or in any special place, of undifferentiated mesenchyme cells which can give rise to new angioblasts in the case of repair after destruction of tissues.

In the living chick-blastoderms, I have some specimens of the fourth day of incubation in which there are signs that new angioblasts are still differentiating in the membranes; the chicks themselves have not yet been studied from this point of view. The work of E. R. Clark indicates very strongly that there comes a time in normal growth when the differentiation of new angioblasts ceases. Thus it is clear that the next logical step is to test the question in adult tissues as to whether in the repair of injury all of the new growth of vessels is from preceding endothelium or whether there may be undifferentiated cells in the mesenchyme that may form new angioblasts. It was for this reason that the opportunity to study the healing in intestinal wounds with Dr. Halsted and Dr. Holman was so attractive to me.

The evidence from this study is that, in the healing of intestinal wounds in the adult dog, all of the new growth of vessels is from preceding endothelium. The question has still to be attacked in certain specific areas like the spleen and bone marrow, where the organs are built upon the vascular system in a manner different from that in other organs.

As in the case of the mucosa, the striking point about the Halsted operation is the minimum amount of destruction of tissue and consequently the minimum of repair. This is as characteristic of the vascular system as of the mucosa. In fact the viability of the mucosa is dependent on the lack of injury to the circulation.

Through the work of Mall the vascular system of the dog's intestine is well known. The blood-vessels can best be studied by making complete injections and then observing the results either in thick sections or by stripping the coats apart as membranes. The entire specimen should be fixed in formalin and then placed in 70 per cent alcohol; the coats can best be stripped apart while in 70 per cent or 80 per cent alcohol. Both sections and membranes should then be dehydrated and cleared by the Spalteholz method, which is a very great addition to the technique of studying vascular problems.

One of Mall's great contributions was to show that the vascular patterns in organs are very exact and constant. For the intestine he pointed out that if the superior mesenteric artery is to be regarded as an artery of the first order, the radiating branches in the mesentery are of the second, the arches are of the third, the series of long, penetrating arteries for the submucosa are of the fourth, while the short arteries from the secondary arches are of the fifth order.

In following the repair of the vascular system, thick sections such as the one in Fig. 18 are cut through the anastomosis: or the wall is stripped apart and cleared by the Spalteholz method, and the pattern is then compared with the normal pattern. In this way one can say with precision that this or that area is a zone of new vascular growth. Thin sections from adjacent blocks can then be studied histologically. I should like to stress the importance of this method as giving precision to the attack of any problem involving a new growth of vessels; that is, the necessity of starting with a knowledge of the normal pattern of the vessels and a method of determining the exact areas of deviation from the normal.

In Fig. 18, which is a thick section adjacent to the one in Fig. 6, there are three striking points in connection with the blood-vessels; first, that the pattern of the vessels of the muscularis, the submucosa and the mucosa, is normal; second, that there is a marked dilatation of both arteries and veins of the submucosa, and a similar dilatation of the capillary plexus around the crypts of Lieberkühn in the mucosa; third, that there is a marked new growth of vessels in the area of the apposed serosal coats. At the very apex of the anastomosis the mucosal vessels are, of course, new and their pattern is unusual, one very large straight vessel being especially conspicuous. The black area just beneath the apex is a suture about to be cast into the lumen of the bowel like the one in Fig. 6.

The dilatation of the vessels of the submucosa and around the crypts in the mucosa is constant; it occurs as early as the first day after the operation, as shown in Fig. 1, in which it is very striking in the basal part of the mucosa. In the mucosa it involves not only the arteries and veins but also the capillary plexus. The amount of dilatation of the plexus of the mucosa in this case (Fig. 1) is strikingly brought out in Fig. 2, which shows a part of the same section taken at the same magnification, the vessels being equally well injected but too small to show in the photograph. A thicker section from this specimen brings out the same dilatation in the vessels of the submucosa. The dilatation persists until late; it is present in all of the specimens up to about 50 days when it is overcome. This dilatation must be either a local functional adaptation of the vascular system or is due to a paralysis of the smooth muscle of the vessels.

In the main the growth of vessels is not general but is limited rather to specific areas. The two constant places are the apposed serosal surfaces, as seen in Fig. 18, and the apex of the mucosa, shown very well in Fig. 20. Otherwise, a new growth of vessels is associated with some accidental occurrence, such as an adherent omentum, a thickening of the serosa near the anastomosis, the organization of the wall of a stitch abscess or certain stresses on the tissues connected with the pulling out of the stitches. The most interesting place to study the regeneration is in the apposed serosal surfaces because it is here that the vessels, old and new, can be

studied with the greatest precision. It is necessary first to know the normal blood supply of the serosa. Besides the penetrating arteries, long and short, which course through it, the serosa normally has no vessels that are particularly its own, but certain long capillaries, belonging to the external longitudinal muscle coat, lie on the outer surface of the muscle, hugging it very closely and serving as serosal capillaries in any abnormality of the serosa. That is to say, some of the long capillaries characteristic of smooth muscle lie within the longitudinal muscle, some on its outer surface. This is well shown in Fig. 2. It is these capillaries which are most involved in this new growth, a point well brought out in two figures from a specimen 4 days after the operation. Fig. 16 shows one half of the interned flap, the other having separated in cutting the block. Along the lower border are seen the new vessels of the serosa, while in Fig. 17 is the actual interned serosal margin dissected from a piece of the same block. Of course this piece includes the longitudinal muscle coat, and if it were from any other part of the intestine it would show simply the well known normal pattern of long capillaries characteristic of smooth muscle. It is thus obvious that by the fourth day there has been a very extensive new growth of vessels in this area all along the interned serosal margin.

The interesting point in regard to this new growth is as follows: In the stages beginning at the second day and extending to the third, fourth, and fifth days after operation, there is a very marked proliferation of the endothelium of these serosal capillaries, as well as of the deeper capillaries of the muscle coats. This same proliferation also takes place in the small arteries and veins, certainly in those of the sixth order. The endothelium of the capillaries and of the vessels of the sixth order, both arteries and veins, returns therefore to the embryonic type of angioblastic masses, with the same characteristic crowding of nuclei, in the zone of stimulus. It would certainly be interesting if one could determine what the stimulus is, most possibly a chemical one; it certainly acts in very specific areas. The old endothelial cells divide until they appear as shown in Figs. 21 and 22, the first one showing such an artery and vein while the second shows a capillary. Fig. 21 is taken from the serosal margin of the case shown in 16, while Fig. 22 is from the interned muscularis. It will be seen that the endothelial nuclei are larger then normal and almost touch each other, while the wall as a whole looks swollen. The nuclei of the capillary look smaller in comparison simply because the drawing is less magnified. In Fig. 21 the lower vessel is the artery, shown by the long transverse nuclei of muscle in the lower part and the row of muscle cells in transverse section in the left; the piling up of the nuclei is perhaps even more striking in the vein. Exactly the same heaping up of nuclei is to be seen when the vessels are cut in cross section, so that there must be an almost continuous layer of endothelial nuclei all around the wall of the vessels. Mitotic figures are easy to find in these stages.

This preliminary division of the endothelial nuclei is the first step in the regeneration of vessels. The second is shown in Fig. 23 from the infolded serosa of the same case. This represents the new growth, a solid mass of angioblasts from an old vessel of embryonic type. In this figure the old capillary is within the longitudinal muscle and has been injected, as is shown by the black granules of the ink, while from its end is a solid mass of cells which have just reached the serosal margin from which the mesothelial cells have disappeared. There is a mass of fibrin, containing a few cells, at the upper left corner of the figure. The solid mass of angioblasts is just beginning to show a vacuolization of the cytoplasm, like that in the embryo, to form a new lumen. Thus the return of the vessels to the embryonic type is complete; the endothelial cells first divide, then return to the angioblastic type, then give rise to masses of new angioblasts which grow out to form new vessels. These new vessels are at first capillaries, and from these capillaries arteries and veins differentiate.

In one of our cases injected on the fourth day after the operation, we tied off two of the long vessels of the mesentery in order to test out the time when there is an anastomosis of new vessels from one interned flap to the other. The experiment proved that the connection is partial on the fourth day. It is maximal on the eighth or ninth, the fusion being then complete. The mass of angioblasts in Fig. 23 has, however, not yet joined with similar angioblasts of the other flap, nor has the lumen become complete; but such a union of the two masses of angioblasts and the opening of the lumen through these masses of cells, would complete the vascular connection of the two sides. It will be remembered that one of the most characteristic of the properties of angioblasts is their tendency to join similar masses of cells by the process of sprouting to form plexuses.

Thus the process of the formation of new vessels has been followed completely in this one place, for on the first day no change can be seen in the serosal margins, on the second day there is a marked multiplication of the endothelial nuclei of the old vessels—that is of the capillaries and of the small arteries and veins—while by the fourth day there is a growth of new angioblasts from the altered endothelium of the old vessels, some of these masses having developed a lumen and joined with similar masses of the other side. By the ninth day the union of the two sides by new vessels is complete, and there is an extensive pattern of new vessels along the entire infolded serosal margins. In the case of the dog which had a peritonitis, the walls of the anastomosis were infected, and it is interesting to note that these processes of regeneration of the vessels were much retarded.

In Fig. 24 is given further evidence that the lumen of the new vessels arises by the vacuolization of angioblasts. This is from a new vessel which is becoming an artery and has been completely injected, but which shows a lumen as irregular as it can possibly be. Large masses of ink in the lumen are connected with other masses by channels so tiny that only single granules of ink can get through them. No such pattern is ever found in old vessels, the type of lumen in which can be seen in Figs. 21 and 22, but such a pattern would be exactly simulated if the developing vessels of the early chick blasto-

derm, such as are shown in Fig. 23 of my paper in the Mall Memorial Volume (Sabin, 1920) were injected. On the right of Fig. 24 is a vessel cut in cross section showing the same heaping up of the nuclei of the endothelium, while in the center is the edge of an artery showing simply the muscle cut tangentially and not involving the lumen at all. In studying an area showing a large number of new capillaries it is very striking that one can find many capillaries in which the lumen is excessively narrow, allowing but single rows of granules of the ink to pass through them, as in Fig. 24, and this appearance is due, I think, to the fact that the lumen is formed, as in the embryo, by a vacuolization of the cytoplasm. The interpretation of this process could hardly be made from sections and was made by watching the phenomenon in the living chick and following a single mass of solid angioblasts through this process of the liquefaction of the center of the mass to the formation of a vessel lined with endothelium.

There is of course difficulty in getting the evidence from sections to say that not a single angioblast arises independently from the mesenchyme and one must first study with care all of the other types of cells involved. They are of course numerous. Around the abscesses are very beautiful examples of the clasmatocytes or the resting wandering cells of Maximow, also known as the pyrrhol cell of Goldmann and belonging to the class of the macrophages of Evans. In the regenerating tissue, that is to say, in the active phase, these wandering cells are very characteristic, being filled with round vacuoles and showing all the signs of phagocytosis rather than presenting the type of exceedingly irregular vacuolization shown by angioblasts. Secondly, the clasmatocytes are not multinuclear. In point of fact the only cell that can be confused with new angioblasts in this tissue is the giant cell associated with foreign bodies. Such a giant cell might be difficult to differentiate from a mass of angioblasts and I found one which closely simulated the wall of a new vessel. It was long and narrow, one of its nuclei was in mitosis, and it looked much like a chain of angioblasts; it could be shown, however, to be a giant cell, as it was close to one of the stitches, was surrounded by several characteristic giant cells, and what was more important, it showed active phagocytosis, there being two red blood corpuscles engulfed in its cytoplasm besides a large amount of acidophilic debris. Thus, though there may be difficulty in saying that there is not a single newly differentiated angioblast in the material, one may say that no cells looking like angioblasts and wholly disconnected from the old endothelium have been found. Moreover, the marked preliminary transformation of endothelium of the old vessels returning to the angioblastic phase is strong evidence that the new endothelium comes from the old.

The point which seems to me to be of the greatest interest in connection with the new growth of vessels is, that in the zones which are proved to be specific areas of new growth the vessels return to their angioblastic phase and the new angioblasts grow out as solid masses of cells in which a lumen is formed just as the lumen of the vascular system is formed in the embryo by an intracellular process of vacuolization.

In Figs. 19 and 20 are shown the ultimate result of the healing of the vessels. The depression at the left of Fig. 19 is the apex of the anastomosis where the vessels have now returned entirely to the normal pattern, the dilatation being wholly overcome. On the other hand, there has been a marked new growth of vessels in the submucosa throughout the center of the section, due to the stretching of the submucosa by the pulling out of a stitch which now shows within the muscularis to the left of the large vessel crossing the circular muscle coat. There is in all of the anastomoses a considerable alteration from the normal pattern of the vessels of the wall due to a partial isolation of patches of muscle where the continuity has been broken by the pulling of the sutures. In the other section (Fig. 20) there is a striking thickening of the serosa along the border of the section and the apex of the anastomosis to the right is marked by a very unusual vascular pattern. Just beneath the apex is a zone where the muscle has not regenerated and to the left a second such layer of connective tissue containing a very large vessel passing to the submucosa. Both specimens show that the dilatation of the vessels, so marked in earlier stages, has been in the main overcome.

Conclusions

In these studies it has been shown that in the end-to-end anastomosis of intestine the infolding of the wall does not produce any blocking of the lumen, that the infolded parts are viable, and that the mucosa continues to function in absorption, but that there is a very great increase in the number of cells secreting mucus. Ultimately this change in functional adaptation is brought back to the normal by the death of the mucus cells and the regeneration of the mucosa.

It has been shown that wherever the muscularis mucosæ is cut or ruptured without the occurrence of an infection, the glands of the mucosa invade the submucosa, often forming cysts there, but that these glands are subsequently restored to the mucosa by the regeneration of the muscularis mucosæ which makes a barrier for the glands.

For the smooth muscle, the histological technique shows that its cells are very sensitive to injury and that it is hardly possible to put a clamp on smooth muscle without producing changes that can be recognized under the microscope. It seems to me a very important point that our series of end-to-end anastomoses have produced no dilatation of the wall of the intestine and hence no damage to the musculature sufficient to make it thin out. All of the cases show gaps in the musculature where the stitches pull through, but though this damage is inevitable, the fact that the muscle is syncytial, and that the stitches are limited to six points around the lumen of the gut, seems to limit the amount of destruction to the musculature more than in the end-to-side form of the operation.

In the regeneration of the vascular system it has been shown that the regeneration of the vessels is limited to specific areas where the growth of new vessels is preceded by a change in the endothelium of the old vessels which may be characterized as a return of the endothelium to the original angioblastic type

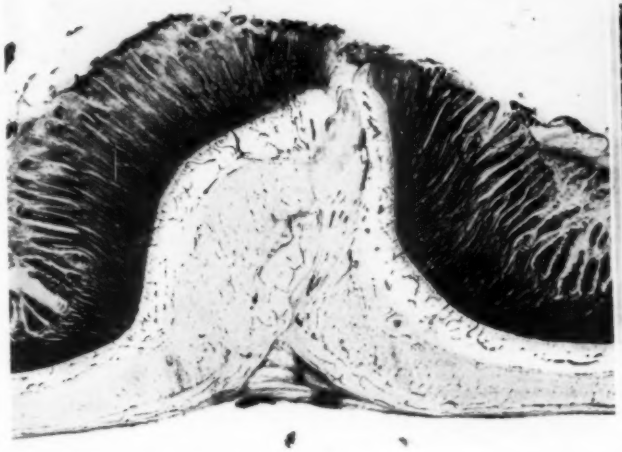

Fig. 1

Fig. 2

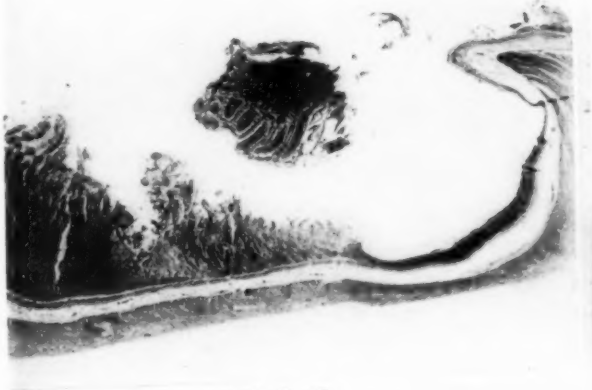

Fig. 3

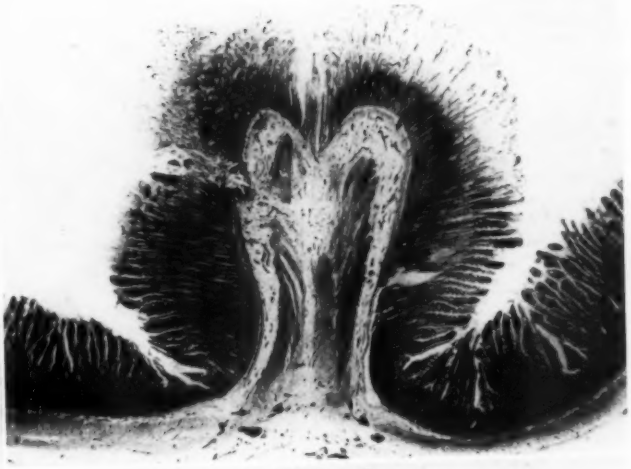

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

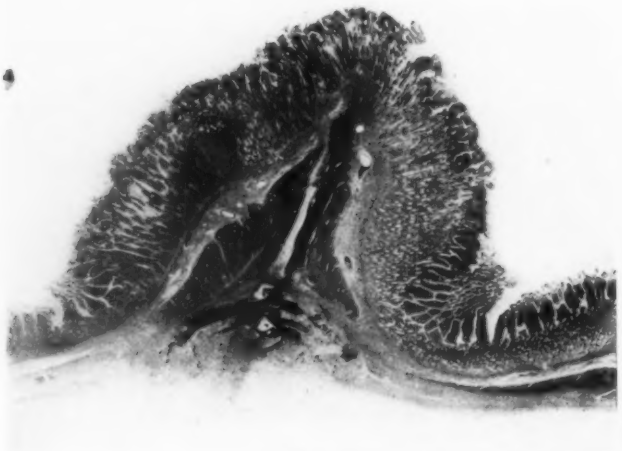

Fig. 11

Fig. 12

Fig. 14

Fig. 19

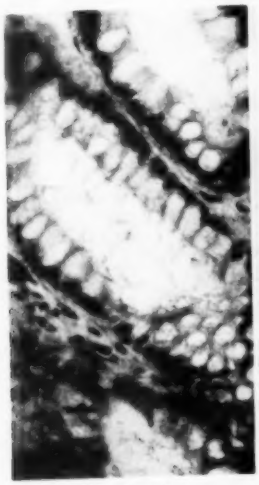

Fig. 13

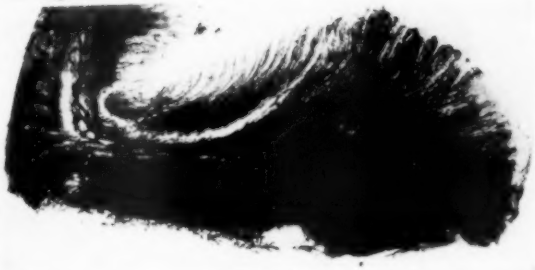

Fig. 16

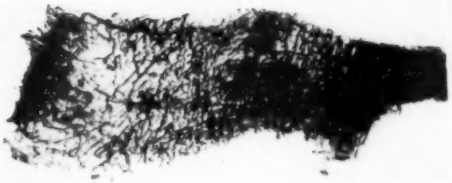

Fig. 17

Fig. 15

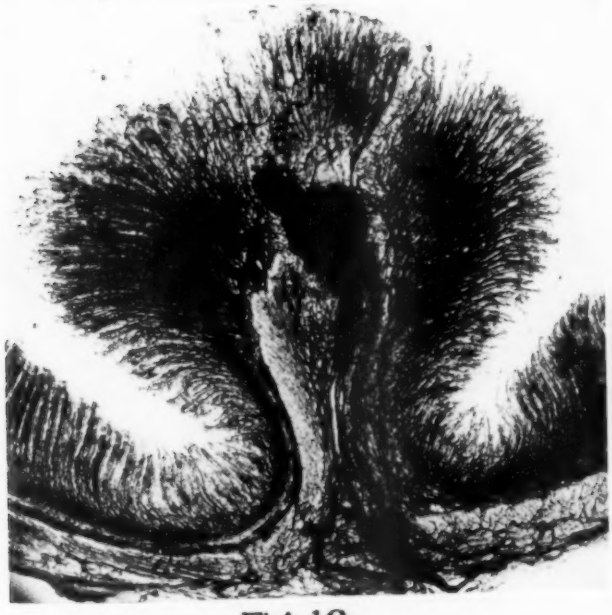

Fig. 18

Fig. 20.

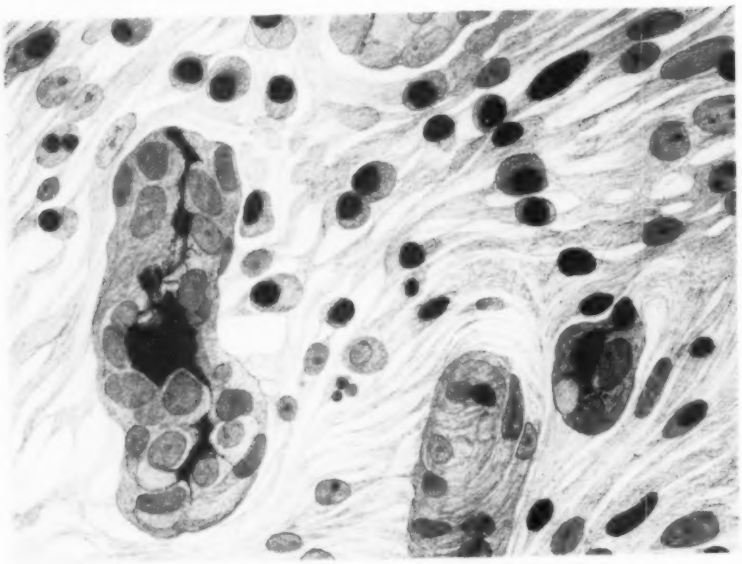

Fig. 24

Fig. 22

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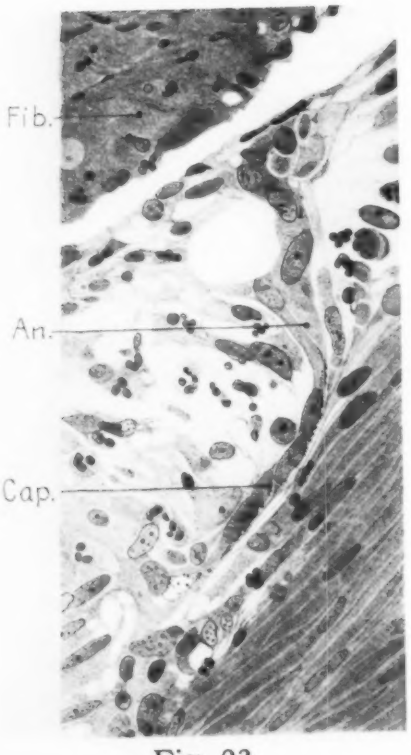

Fig. 23

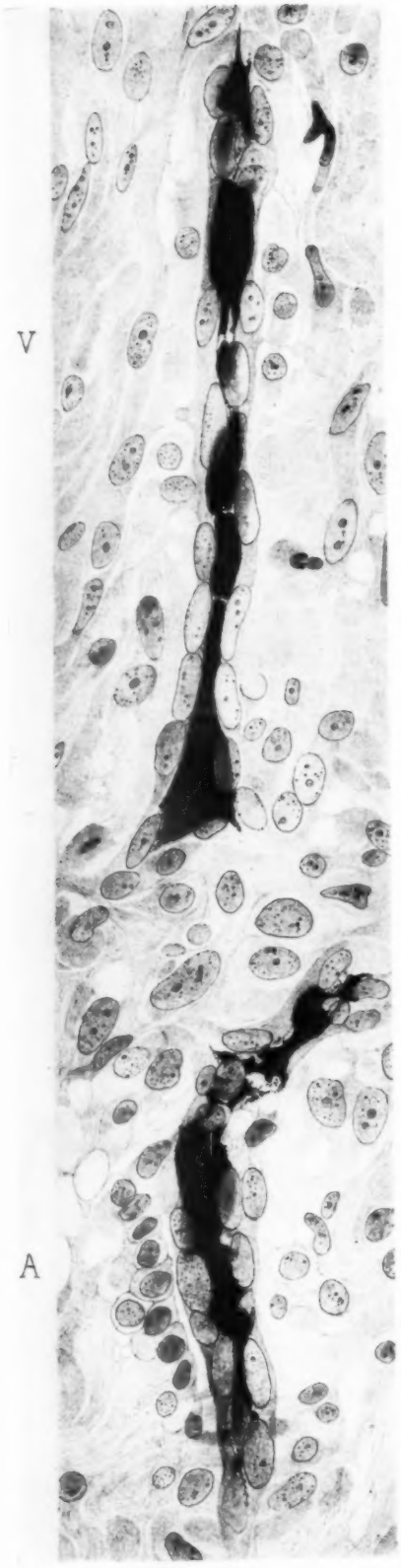

Fig. 21

involving a very great multiplication of the endothelial nuclei, and that from these transformed vessels solid masses of angioblasts like those of the embryo grow out, acquire a lumen, as in the embryo, through liquefaction of the cytoplasm and become new vessels, first capillaries, then arteries and veins.

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DESCRIPTION OF PLATES

FIG. 1.—Section through an anastomosis in the jejunum of a dog (No. 5) injected with India ink 21 hours after operation. It shows an excellent union by fibrin and a marked dilatation of the mucosal plexus of vessels. Stained in cochineal. 100μ. × 10.

FIG. 2.—Part of the same section as Fig. 1, to show that the undilated mucosal plexus is not visible at this magnification. × 10.

FIG. 3.—One end of the intestine after an operation in which a Murphy button had been used. The dog (No. 13) was killed immediately after the operation. It shows a marked destruction of the wall. H and E. 15μ. × 10.

FIG. 4.—Section through an anastomosis in the jejunum of a dog (No. 9), injected with India ink 9 days after operation. It shows the complete regeneration of the glands of the mucosa at the tip of the anastomosis. H and E. 10μ. × 10.

FIG. 5.—Section through an anastomosis in the duodenum of a dog (No. 2), injected with India ink 14 days after operation. It shows the regeneration of the glands and the beginning of the new villi at the tip of the anastomosis. H and E. 15μ. × 6.

FIG. 6.—Section through an anastomosis in the jejunum of a dog (No. 19), injected with India ink 16 days after operation. It shows a stitch in the process of passing into the lumen of the bowel and a downgrowth of the glands opposite a place where the muscularis mucosæ was ruptured. It is from the same case as Fig. 18. H and E. 15μ. × 8.

FIG. 7.—Section through an anastomosis in the duodenum of a dog (No. 3), taken 22 days after operation. It shows a practically perfect recovery. At the left is a downgrowth of glands opposite a rupture of the muscularis mucosæ. H and E. 10μ. × 10.

FIG. 8.—Section through an anastomosis in the duodenum of a dog (No. 5), taken 25 days after operation. It shows a downgrowth of glands opposite a rupture in the muscularis mucosæ. H and E. 10μ. × 10.

FIG. 9.—Section from the same case as Fig. 8, to show the development of a compound lymph gland opposite a rupture of the muscularis mucosæ. H and E. 10μ. × 10.

FIG. 10.—Section through an anastomosis in the jejunum of a dog (No. 14), 38 days after operation. It shows a deep downgrowth of glands at the apex of the anastomosis. Fig. 14 is a small area of the same section. H and E. 15μ × 10.

FIG. 11.—Section from an anastomosis in the jejunum of a dog (No. 15), 50 days after operation. It shows a suture still in place holding the infolded parts symmetrically. H and E. 15μ. × 9.

FIG. 12.—Section through an anastomosis in the jejunum of a dog (No. 11), injected with India ink 69 days after operation. A suture still holds one flap infolded and the section shows the crypts transformed entirely into mucus glands. H. and E. 15μ. × 8.

FIG. 13.—Glands from the mucosa of the section in Fig. 7, to show how complete the transformation into mucus cells has been. Dog (No. 3), 22 days after operation. 10μ. × 310.

FIG. 14.—Group of glands shown in Fig. 10, magnified to show the development of a new muscularis mucosæ. Dog (No. 14), 38 days after operation. H and E. 15μ. × 40.

FIG. 15.—Section through the infolded musculature of an anastomosis in the jejunum of a dog (No. 12), injected 25 hours after operation, to show the damage to the smooth muscle. H and E. 10μ. × 60.

FIG. 16.—Section of one of the infolded flaps of an anastomosis in the jejunum of a dog (No. 8), injected 4 days after operation. Thick free-hand section, cleared by the Spalteholz method. It shows new vessels along the interned serosal margin. × 10.

FIG. 17.—Piece of the interned serosa and longitudinal muscle dissected off from a block of the same specimen as Fig. 16, to show the new growth of vessels.

FIG. 18.—Section of an anastomosis of the jejunum of a dog (No. 19) injected with India ink 16 days after operation. Same case as the one shown in Fig. 6. Thick free-hand section cleared by the Spalteholz method. × 12.

FIG. 19.—Section of an anastomosis made in the jejunum of a dog (No. 5), injected with India ink 25 days after operation. Thick free-hand section cleared by the Spalteholz method. From the same anastomosis as the one shown in Figs. 8 and 9. It shows a suture which, in pulling out of the submucosa, has produced a stretching and a consequent new growth of vessels. × 5.

FIG. 20.—Section of an anastomosis made in the jejunum of a dog (No. 15), injected with India ink 50 days after operation. Free-hand section cleared by the Spalteholz method. Same specimen as the one in Fig. 11. × 12.

FIG. 21.—Drawing of an artery and a vein in the interned serosal border of an anastomosis in the jejunum of a dog (No. 8), injected 4 days after operation. It is from the same case as the one shown in Fig. 16. It shows the multiplication of endothelial nuclei which is the first step in the regeneration of vessels. A, artery; v, vein. H and E. 10μ. × 700.

FIG. 22.—Drawing of a capillary in the interned musculature of the same case as Fig. 21 from dog (No. 8), to show the same multiplication of the endothelium preliminary to the growth of new vessels. H and E. 10μ. × 525.

FIG. 23.—Drawing of angioblasts extending from an injected capillary in the longitudinal muscle to the serosal margin, from the same case as in Figs. 21 and 22. Dog (No. 8). *An.*, angioblasts; *cap.*, injected capillary in the edge of the longitudinal muscle coat; *fib.*, fibrin becoming organized between the two serosal surfaces. H and E. 10μ. × 700.

FIG. 24.—New blood-vessels in the serosa adherent to the omentum in an anastomosis of the jejunum of a dog (No. 4), injected with India ink 7 days after operation. The extreme irregularity of the lumen of the vessel to the left shows it to be a new vessel. The injected vessel to the right shows the heaping up of the endothelial nuclei in cross section. H and E. 10μ. × 1270.

END-TO-END ANASTOMOSIS OF THE INTESTINE BY PRESECTION SUTURES

AN EXPERIMENTAL STUDY

By EMILE HOLMAN

(From the Surgical Department, The Johns Hopkins University)

Since the days of the "four masters" who lived in the thirteenth century and who pronounced the dried trachea of the goose to be the most suitable and useful adjunct in the repair of the ruptured bowel wall, intestinal suture has been a fascinating study to surgeons, replete in the words of Senn with "stupendous ignorance, clever mechanical ingenuity and patient experimental research."

The following study was undertaken in collaboration with Dr. Sabin at the suggestion and with the generous help and advice of Dr. Halsted. It was our intention to study the regeneration of blood-vessels between the apposed serous surfaces in an intestinal anastomosis, but in the course of Dr. Sabin's careful observations other very interesting details were noted and have been recorded by her in the accompanying article. Similarly, in the development of our operative procedure, modifications were frequently made, resulting finally in a form of end-to-end anastomosis referred to throughout this article as the "presection type." So many and varied have been the methods of intestinal anastomosis described, that it is almost with an apology that this method is offered, but it is given in the hope that it may furnish certain suggestions which may be of value to others in the perfection of an end-to-end anastomosis which will merit adoption.

With the development of intestinal suture have come certain well-defined principles deserving common recognition, but more frequently sinned against than observed in the methods of circular suture now in current use. Briefly stated these principles are:

1. The necessity of depending on an approximation of peritoneal or serous surfaces for a firm and permanent closure. It was in 1802 that Bichat in his *Traité des Membranes* called attention to the absence of adhesions between mucous surfaces and their very frequent occurrence between serous surfaces, but it was not until Lembert's and Jobert's work in 1826 that this observation was applied to intestinal suture, and insisted upon as the *sine qua non* of intestinal repair.

2. The necessity of including in one's stitches a bit of the submucosa or fibrous coat of the intestinal wall, as pointed out by Dr. Halsted in 1887. Textbooks of surgery (*vide* Gould's *Operations on the Intestines*) still speak of "seromuscular" stitches, whereas it has been sufficiently demonstrated that such stitches are utterly useless and dangerous, unless they include also a bit of the submucosa.

3. The avoidance of soiling the field of operation by the escape of intestinal contents or by the handling of the mucous coat of the bowel.

4. The necessity in end-to-end union of maintaining the blood supply intact to the edge of the divided intestine. The fear of ischemic necrosis of the inturned portion due to pressure on the mesenteric vessels has no doubt been the greatest deterrent factor heretofore in influencing surgeons against the end-to-end suture.

5. The elimination of trauma in performing any intestinal surgery.

These principles have been well observed in the procedures adopted in The Johns Hopkins Hospital for gastro-enterostomy and lateral anastomosis, which is the method most commonly employed clinically in intestinal suture. With particular reference to the last point, Dr. Halsted has long since insisted on the avoidance, in gastro-enterostomy, of approximation clamps which are so liable to inflict injury to the bowel wall. Traction sutures are employed instead, which permit even greater facility in the application of sutures than that afforded by the use of clamps.

With reference to intestinal anastomosis, the great desirability of end-to-end union as compared to lateral anastomosis has been demonstrated so conclusively by the excellent studies of Cannon and Murphy, that we were prompted in our experimental work to employ the circular suture, and so far with excellent results. In 42 end-to-end approximations performed in this laboratory, two subjects developed peritonitis, but both were killed before death occurred. In fact one was killed eight days after the operation, no trouble having been suspected, since the dog was eating normally. All others were sacrificed in good condition, or died of other causes, as, for example, injuries following a fight or an intussusception entirely independent of the anastomosis. Ashton and Baldy recorded a mortality of 30 per cent in their end-to-end approximations and were firmly convinced that only lateral anastomosis should be attempted on the human subject.

Cannon and Murphy's studies were most instructive with reference to the progress of food through the intestine after

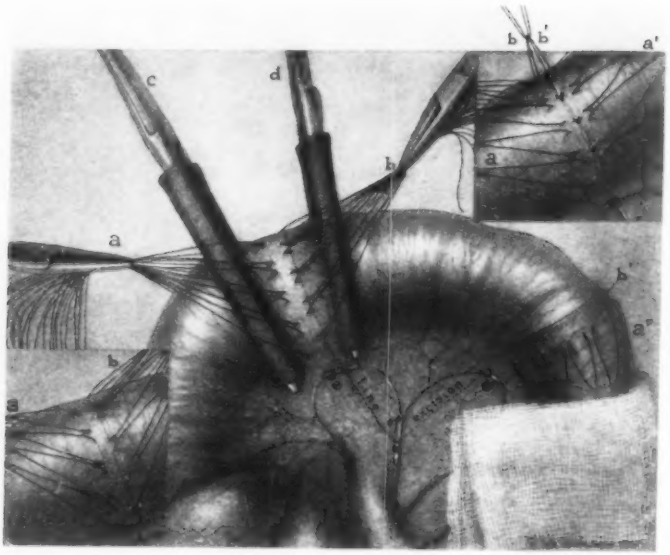

FIG. 1.—Drawing illustrating the method of placing the sutures in the presection type of end-to-end anastomosis of the intestine.

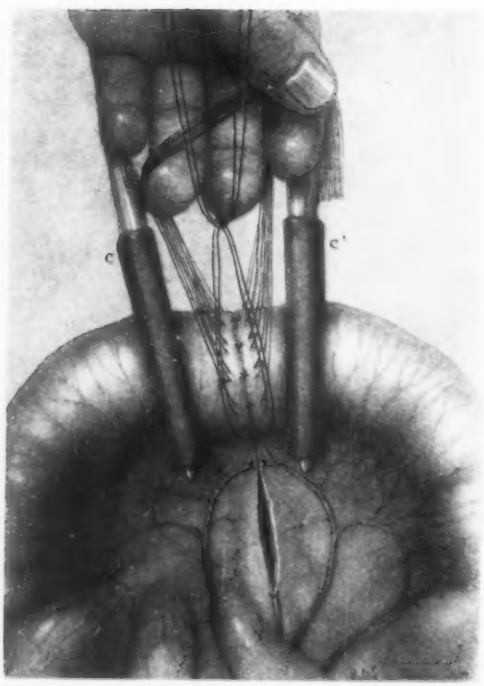

FIG. 2.—Drawing made after the first row of sutures, *b* and *b'* of Fig. 1 have been tied to approximate the mucosal surfaces.

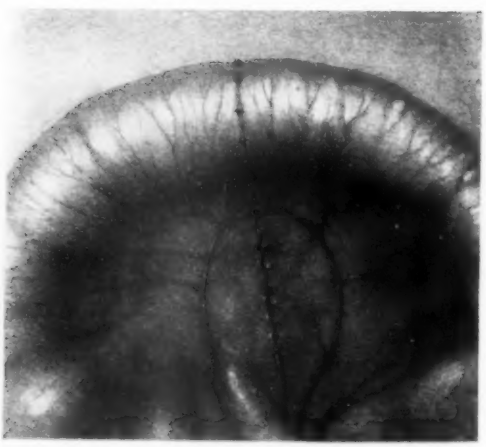

FIG. 3.—Drawing made after the second row of sutures, *a* and *a'* have been tied to approximate the serosal surfaces.

lateral and circular sutures. The experiments were performed on cats with an obviously small lumen of the intestine, and fluoroscopic observations were made following the ingestion of bismuth meals on the first, second, fourth, seventh, and tenth day after the operations.

In no case of end-to-end union was there the slightest evidence of stasis of food in the region of the operation to be observed. The results were quite different with lateral anastomosis. As long as food was passing through the intestine some of it was always present at the point of junction, and when almost all the unabsorbed material was in the colon there still remained a large mass filling the widened lumen where the coils were laterally joined. Observation the next day showed the mass still at the anastomosis. . . . . Inasmuch as stasis of the food was not observed at any time after end-to-end union of the severed gut, while after lateral anastomosis the ordinary food was stagnant in the region of the junction, it is clear that, other things being equal, the end-to-end union is to be preferred to the lateral for rapid resumption of the normal functioning of the canal.

In confirmation of these observations circular and lateral sutures were performed on Dog 18, who was killed in a fight 4 months and 6 days after the operation. The specimen obtained was a most interesting one: the circular suture was located proximal to the lateral suture, and was unaccompanied by even the slightest dilatation proximal to the small diaphragm resulting from the inturned bowel. At the point of the lateral anastomosis, however, there was marked bulging of the two blind ends and in spite of an opening 5 cm. long, there was considerable hair and a small marble-sized bit of bone lying in the proximal end, detritus which had passed through the circular suture above. To quote Cannon again:

As to the claim made for lateral anastomosis that it permits the opening between the two intestinal ends to be as large as one may wish, it may be stated that this can be done only by more extensive cutting of the circular muscle of the intestine, thereby still further interfering with peristaltic activity, and also, that the condition to be desired is not such a large opening as an opening that functions satisfactorily.

An interesting observation in this specimen was the absence of de-inversion along the line of suture—the persistence of a diaphragm even after four months. Mall and Halsted, and Grey, noted a straightening of the line of suture beginning as early as the fourth week, resulting finally in complete obliteration of the inturned diaphragm.

Cannon also produced definite evidence of the deterrent influence of trauma and rough handling of the bowel upon the progress of food. Whereas prolonged etherization and cooling or drying of the bowel produced no inactivity of the alimentary canal, manipulation of the stomach and intestine, even gently and under most favorable circumstances, resulted in the absence of any movements of the stomach, with no discharge of food into the intestine, for three full hours after the feeding. Normally a discharge of food from the stomach begins within 20 minutes after ingestion.

Further evidence of the value of gentle handling and lack of trauma is furnished by Dr. Sabin's observations and illustrates well the importance of greater coordination and cooperation between anatomist and surgeon in the interpretation and improvement of surgical experimental work.

Five types of end-to-end anastomosis were performed—in certain instances two kinds in the same dog—but the method finally determined upon as the most satisfactory is the one described as the "presection type."

In Dogs 1, 2, 3, 4, and 5, circular anastomoses were performed by means of Halsted mattress sutures reinforced with Lembert sutures and, although excellent specimens were obtained, these sutures were all placed following the division of the bowel, and therefore with more likelihood of contamination of the operative field. In Dogs 6 to 25 anastomoses of the presection type were performed, in certain cases as high as three sutures in the same dog. In Dogs 12 and 13 anastomoses of the Connell type were also performed. In this type the sutures are placed through all the coats of the bowel and tied within the lumen. Observations recorded at the time of the operation were quite unfavorable as compared with the presection type: the septic interior surface of the bowel was being handled constantly, making asepsis impossible, and the last half of the bowel was sutured with considerable difficulty, due to the eversion of the mucous membrane and to the difficulty of handling the bowel after division.

In Dogs 3, 12 and 19, anastomoses of the plain end-to-end type were performed, similar to that described by Moynihan, in which a running suture through all the coats of the bowel is taken approximating the mucosa, with a second continuous suture approximating the serosa. The same objections apply here as to the Connell type, with reference to the septic surfaces handled, and the difficulty of placing stitches after division of the bowel.

In Dog 13 circular union by means of the Murphy button was established. As early as 1893, Senn stated that any "instrument, suture, or ligature used in effecting the continuity of divided bowel that produces gangrene must be looked upon as a source of danger." As shown by Barbat, the Murphy button is characterized by the sloughing of the inturned portion with ulcer formation, facts sufficient to condemn it, aside from the confirmatory histological observations of Dr. Sabin.

The procedure employed in the presection type is as follows: The two points of proposed division of the bowel are selected at any distance apart, preferably where the vessels of supply are easily seen. The loop to be resected is milked free of intestinal contents, and rubber-covered clamps applied *very gently* and with but just sufficient pressure to prevent the passage of intestinal contents. The clamps are applied on each side of the two lines of division about 1 inch removed from the line of division.

The loop arteries paralleling the bowel are ligated at points e and e', exactly opposite the proposed lines of division. The row of a sutures is then applied $\frac{1}{4}$ to $\frac{3}{16}$ inch removed from the proposed line of division, the first suture being applied at the mesenteric border, and the last on the opposite side also at the mesenteric border. Occasionally, the two may be seen to overlap, thus insuring an adequate approximation at this criti-

cal point. The sutures are applied at right angles to the lumen of the bowel and parallel to the larger vessels coursing over the intestine. They include about $\frac{1}{2}$ inch of bowel wall with a bit of the submucosa, and are placed about $\frac{3}{16}$ inch apart, until the whole bowel has been surrounded with a line of interrupted sutures, *a*. A second row of presection sutures, "presection" in that they are applied before any incision into the bowel is made, is taken paralleling the *a* sutures and removed from line of proposed division by only $\frac{1}{16}$ inch. These *b* sutures are placed exactly opposite the intervals between the *a* sutures, thus ensuring against any leakage between the interrupted stitches.

Two similar rows of *a'* and *b'* sutures are applied at the second line of proposed division, the number of *a'* sutures corresponding exactly to the number of *a* sutures. If the proximal portion of the bowel is dilated, and the distal portion constricted, one may apply the sutures in the constricted portion in a line at an obtuse angle instead of at a right angle to the bowel, and divide the intestine accordingly, thus increasing the size of the opening in the constricted portion.

The main arteries to the loop of the intestine to be resected are then ligated and divided. If these arteries are ligated at the beginning of the operation, the anemia of the bowel wall causes vigorous peristaltic movements which interfere with the placing of the presection sutures. The bowel is divided as closely as possible to the lines of *b* and *b'* sutures, and the intervening portion excised.

The *b* and *b'* sutures are then tied, the two strands of each suture being held and tied as a single thread, each knot, therefore, being made up of four strands of silk. This first line of sutures gives an accurate approximation of the mucosa. The second lines of *a* and *a'* sutures are tied in similar fashion, the two strands of each suture again being held and tied as a single thread. These sutures give an approximation of the serosa around the entire circumference of the bowel, and at the mesenteric border the two adjoining sutures on opposite sides, with an occasional overlapping, ensure an adequate inturn and firm closure at this important point. The rent in the mesentery is closed with continuous or interrupted black silk sutures, care being taken not to include in the stitches any of the vessels supplying the united loops of intestine.

The points of importance to be noted in connection with this suture are:

1. All sutures are applied before incision into the bowel, regardless of the amount of bowel to be resected. The value of this is evident from the point of view of asepsis and from the standpoint of facility in introducing sutures before division of the intestine.

2. All sutures are applied without entering the lumen and without handling the septic mucosa at any time. The method has been applied in the large bowel and in several end-to-side anastomoses in the cecum with excellent results and without subsequent infection.

3. The sutures are applied at right angles to the lumen of the bowel and therefore parallel to the large vessels coursing through the bowel wall. Accordingly, none of the larger vessels are constricted when these sutures are tied and, as shown by the injections, there is not the slightest impairment of circulation to the severed end of the various intestinal layers. Furthermore, the villi of the mucosa continue to function to the very edge of the divided bowel, as demonstrated by Dr. Sabin in Dog 25. There was also no evidence in a single specimen of sloughing of the inturned edges, ideal and rapid healing at the point of union being thus provided for.

4. In the entire series there was no evidence of dilatation proximal to the anastomosis, thus indicating the absence of any obstructive narrowing of the lumen of the bowel. If this is true for the intestine of the dog with its small lumen, no such difficulty need be expected in the intestine of the human subject with its larger lumen.

5. There is a minimum of trauma such as accompanies the use of mechanical devices, or the handling of the bowel edges with forceps as in the plain end-to-end and Connell sutures, in all of which injury to the mucosa is quite unavoidable. Dr. Sabin's observations illustrate very clearly the importance of avoiding trauma, and as these observations also show that this method is by no means devoid of defects, they will act as an incentive to attempt further improvements in our technique.

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AN INSTANCE OF VOLUNTARY ACCELERATION OF THE PULSE

By JOHN T. KING, JR.,

Instructor in Clinical Medicine, The Johns Hopkins Medical School

It is a matter of universal knowledge that acceleration of the pulse-rate commonly attends such psychic states as fear, pain and anger. Whatever may be the ultimate source of the stimuli which arouse the pace-maker of the heart to greater activity in such conditions, it is now well established that the immediate control of the heart's rate lies in a delicate balance of influence between two opposing nerve mechanisms—the vagus cardio-inhibitory nerves and the sympathetic cardio-accelerator nerves. Such a balance of influence might be disturbed by certain psychic states through either a reflex stimulation of the accelerator cardiac nerves or a reflex inhibition of the vagi, particularly of the right vagus.

In contrast to the frequent instances of acceleration of the pulse-rate by emotional states, in which the acceleration is reflex and involuntary, voluntary increase of the pulse-rate is very rare. It is possible that emotional states affect the cardiac nerve mechanism reflexly through their influence upon the body as a whole, rather than purely through the discharge of nerve stimuli from the higher to the lower centers. At any rate, the pulse-rate is strikingly immune from control by the will except in a few isolated cases.

West and Savage * have recently described the fifteenth case of recorded voluntary acceleration of the heart beat. The case reported by these writers was that of a young man whose heart was normal on physical examination and electrocardiography; the subject could voluntarily accelerate his pulse, and the pulse-rate increased by 25 beats per minute following the injection of 0.002 gm. of atropin sulphate. During the strain of voluntary acceleration this subject showed dilatation of the pupils, which has been described in connection with previous cases, and elevation of the blood-pressure. It is not easy, even in a case so carefully studied as that of West and Savage, to state the mechanism by which the acceleration was accomplished: dilatation of the pupils suggests activity of the sympathetic nerves, while marked acceleration from the atropin suggests that the vagus inhibition might be readily lifted.

An instance of voluntary acceleration of the heart beat has recently come to my attention. The subject is a white man, aged 26, single. His general health has always been good, though he has been subject to naso-pharyngeal infections, with lymphatic hypertrophy and glandular enlargement. He has always been an unusually "high strung" individual, excelling in games which require speed and quick muscular control. For many years he has been subject to attacks of uncontrollable thumping of the heart and tachycardia when under any marked emotion. A few years ago he gave up school for several months and rested, by the advice of physicians, on account of what was called valvular heart disease.

When the recent war broke out, he made three unsuccessful attempts to enlist, but was refused enlistment on account of his heart. He was summoned early in the war before his local draft board, from which he was referred to a medical advisory board, where he came to my attention. Examination revealed a normal heart, with marked tachycardia, and cardio-respiratory murmurs. He was a "high strung" man, and, as the tachycardia was apparently due to excitement, he was accepted. He went through the training without trouble and saw active service, including the Argonne Forest drive. After the armistice, he reported to a medical officer for a minor complaint, and a diagnosis of effort syndrome was made. He was returned to this country, trained in camp, and discharged as well.

He consulted me in October, 1919, not for any particular complaint, but because he was concerned about the diagnosis of effort syndrome, which had been made in the army. He was rather nervous, and having given up exercise on discharge from the army, he was somewhat out of "condition."

Examination showed a man organically sound. The reflexes were moderately exaggerated throughout. He could voluntarily move his ears and posterior scalp muscles. There was marked tachycardia during examination and a sinus arrhythmia. On counting the pulse, I noticed that there was a rapid rise in rate. The patient then gave the voluntary information that he could accelerate the pulse-rate by "thinking about it."

This power to accelerate the pulse voluntarily was readily verified. Almost immediately on command to accelerate, the rise in rate sets in sharply. The pupils dilate slightly, the respirations become somewhat irregular and seem a little more shallow. The subject says that he creates no emotional state whatever, and that the rise in pulse is due to a calm voluntary effort. He is conscious of his heart beat when it is accelerated, and he knows without feeling the pulse whether or not his efforts are strikingly successful. As to the mechanism of the acceleration, his only conviction is that his respirations are not perfectly natural during the effort to accelerate. It will be seen below, however, by pulse tracings, that the acceleration was equally marked when the subject held his breath as it was under the usual conditions.

* H. F. West and W. F. Savage: Voluntary Acceleration of the Heart Beat. Arch. Int. Med., 1918, XXII, 290.

Several pulse tracings were made by Dr. Donald R. Hooker. The tracings were made at the brachial artery with the Erlanger sphygmomanometer and cuff. The time markings indicate intervals of one second. During the early readings, the subject was rather excited, and the excitement tachycardia made the voluntary acceleration less striking. There was never, however, a failure to accelerate the pulse promptly when the effort was made. The least acceleration per minute was one rise of 20, and the greatest rise was of 40 beats per minute.

Retardation of the pulse-rate seems in all instances to have been a passive one. It was always possible for the subject to approach the initial rate after his effort to retard, but he was not able to retard below the initial rate. In one experiment the patient was ordered to accelerate, and the high speed was maintained for 17½ seconds. In this case the pulse "got beyond control," because the high rate was held for so long a period, and it fell very gradually when the effort to retard was made. The pulse-rate at the beginning of this experiment was 108; it rose with effort to 131, was sustained for 17½ minutes, then fell to 117 after 20 seconds. After this, the subject suggested that he be allowed to indicate when he would make the efforts to accelerate and retard: this was done, and the results were somewhat more prompt. A characteristic tracing is shown in Fig. 1.

Electrocardiographic tracings were made by Dr. E. P. Carter. The mechanism of the heart-beat was normal throughout.

The results were as follows:

At rest; sitting. Leads 1, 2 and 3: rate 88, rhythm regular; the P-R interval measures 0.12 second.

No. 2: The R-R interval measures 0.56 second (actual rate 107). Following the signal to accelerate, the R-R interval shortens to 0.48 second (rate 125). Following the signal to retard the R-R interval slows to 0.60 second (rate 100).

No. 4: At rest the R-R interval measures 0.72 second (rate 83); following the signal to accelerate, it shortens to 0.60 second (rate 100) and then to 0.56 (rate 107). Following the signal to slow, the R-R interval drops to 0.72 second (rate 83).

No. 5: At rest the R-R interval measures 0.54 second (rate 111). Inspiration, R-R interval measures 0.64 (rate 93). Acceleration, R-R interval shortens to 0.50 second (rate 120). Retardation, R-R interval lengthens to 0.64 second (rate 93). Expiration, sinus arrhythmia.

No. 6: Right vagus pressure. Before, R-R interval measures 0.68 second (rate 88); afterwards, R-R interval measures 0.64 second (rate 93).

No. 7: Left vagus pressure. No conspicuous change.

Comment.—The changes in pulse-rate are not so striking as are those seen in the pulse tracings. The greatest change in pulse-rate by the usual procedure was in No. 4, in which the heart-beats were accelerated by 24 per minute. In No. 5, the effort to accelerate was made while the breath was held in inspiration. The pulse-rate was retarded by 18 beats per minute following inspiration. On effort, with the breath held, the acceleration amounted to 37 per minute, the retardation to 27 per minute. It is apparent, therefore, that acceleration of the pulse was more marked while the breath was held in inspiration than it was under usual conditions.

Pharmacologic tests were not made, owing to the subject's unwillingness to submit to them. He stated that he was very susceptible to the effect of any drug, especially to that of belladonna. Some years ago he was prostrated by taking capsules containing a small amount of belladonna. He is uncertain as to his exact symptoms at that time, but thinks that he suffered from palpitation and a weak pulse.

The explanation of the phenomenon with which we are dealing is not easy and must be purely hypothetical. The evidence at hand is as follows:

1. "High strung" individual, with highly developed voluntary muscle control.
2. Marked emotional tachycardia.
3. History of sensitiveness to belladonna.
4. Statement of patient that the respiration is in some way involved in the process of acceleration.
5. Marked acceleration with the breath held.
6. Sinus arrhythmia.

The last four of these items rather suggest that the acceleration in the individual may be brought about because of an unstable vagus control of the heart beat, allowing the accelerator influences to gain control at times. It is known that sinus arrhythmia is due to rhythmical changes in vagus activity occurring during respiration. Our subject showed marked slowing of the pulse following his holding the breath in inspiration. However, when the effort to accelerate was made, with the breath still held, there was a sharp accelerator response; slowing took place at will with the breath still held. We might interpret these phenomena as signifying: (1) Slowing from vagus following deep inspiration; (2) acceleration from accelerator (sympathetic) activity with the vagus quiescent; (3) slowing from release of accelerator activity; (4) sinus arrhythmia from rhythmic activity of the vagus as the subject resumes breathing. The patient's feeling that his respiratory movements are linked in some way with the process of acceleration and the observed irregularity of breathing suggest that vagus activity may be minimized by such procedure. The patient's history of sensitiveness to belladonna, if reliable, is strong evidence in favor of his having a vagus system which may be readily neutralized, thus allowing accelerator stimuli to gain control of the beat's rhythm.

West and Savage were of the opinion that in their case the voluntary acceleration of the heart-beat was brought about through a primary decrease in vagus inhibition, probably augmented at times by accelerator influences. Although it is difficult to draw conclusions from our case, there is nothing which could conflict with this hypothesis of West and Savage, and all the evidence would tend to substantiate such a conception of this unusual phenomenon.

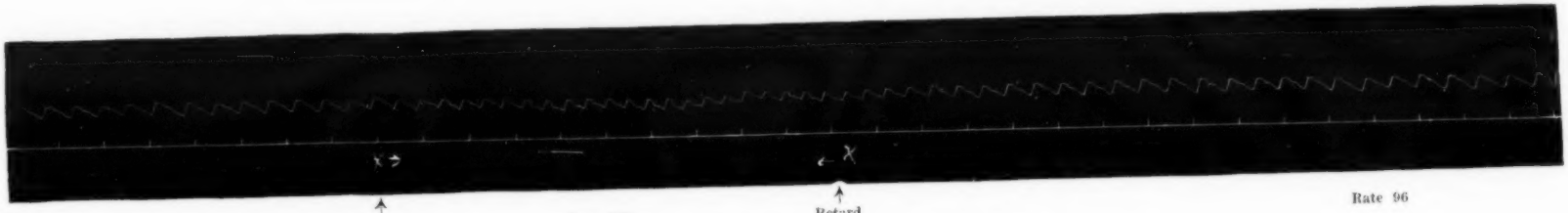

FIG. 1.—Brachial Pulse Tracing. Time intervals 1 Sec.

FIG. 2.—Electrocardiogram (Second Lead). The signals are not reproduced in this print, but the first mark indicates the signal to accelerate, the second indicates the signal to retard.

THE INNERVATION OF THE TENSOR VELI PALATINI AND LEVATOR VELI PALATINI MUSCLES

By ARNOLD RICE RICH

(From the Physiological Laboratory of The Johns Hopkins University)

Gowers began his discussion of the nerve supply of the tensor veli palatini and levator veli palatini muscles with the remark that "the origin of the nerve supply to the palate is one of the most obscure questions connected with the anatomy of the cranial nerves."[1] During a recent study of the physiological relation of the palatal muscles to the Eustachian tube, a review of the literature concerned with the innervation of those muscles disclosed so great a variety of conflicting opinions, clinical observations and experiments that, at the suggestion of Dr. W. H. Howell, it was decided to attempt to study the matter experimentally, since a method had been perfected by means of which the muscles of the palate could easily be exposed, with their nerve supplies intact, for observation in the living animal. It was believed that such a direct method of study would obviate certain difficulties of interpretation which must have hampered many experimenters and clinical observers in their attempts to recognize the movements of the individual muscles while they remained hidden by the mucous membrane of the palate.

The brief outline of the conclusions of previous workers represents an arbitrary selection and by no means exhausts the entire literature reviewed.

1. THE MOTOR SUPPLY OF THE TENSOR VELI PALATINI MUSCLE

The anatomical relationship between the tensor veli palatini muscle, the otic ganglion and the fifth nerve has long been described, and these descriptions, rather than definite experimental evidence, have led the majority of writers to place the motor supply of this muscle in the fifth nerve; for clinical and experimental studies have yielded neither uniform nor satisfying results.

In 1842, Longet[2] described slight movements of the soft palate when he stimulated, in animals, the motor root of the fifth nerve. He assumed that these movements were produced by contractions of the tensor veli palatini, and he therefore placed the motor supply of this muscle in the fifth nerve. Debrou[3] could not detect the slightest palatal movement on stimulation of the fifth nerve, and wrote that "the fifth nerve has nothing whatever to do with the movements of the soft palate." However, he accepted the opinion that the tensor veli palatini is innervated by the trigeminal, and assumed in consequence that the muscle must have very little effect upon the soft palate. Reid[4] had the same experience and wrote, as a result of his experiments on the palatal muscles, that the vagus "is the principal if not the sole motor nerve of these parts"; but he mentioned the fact that a twig from the external pterygoid branch of the fifth nerve to the tensor veli palatini muscle had been described, and he did not exclude the possibility that this nerve might contribute to the motor supply of the tensor. Hein, in 1844, wrote that he had torn away the mucous membrane in freshly decapitated animals and had been able to see contractions of the belly of the tensor veli palatini muscle when he stimulated the motor root of the fifth nerve.[5] The anatomical situation of this muscle makes it difficult to understand how its contractions could be seen with no further dissection than the removal of the palatal mucous membrane.

In 1861, Politzer[6] stimulated the motor root of the fifth nerve within the cranial cavity and observed a dilatation of the pharyngeal orifice of the Eustachian tube on the same side. From anatomical studies he had become convinced that the tensor veli palatini functions as a dilator muscle of the Eustachian tube, and he therefore concluded that this muscle is innervated by the fifth nerve. Turner,[7] on the other hand, vigorously rejected the fifth nerve as the motor supply of the tensor veli palatini and wrote: "I have been unable to find a single recorded case in which disease of this nerve (fifth) was associated with paralysis of the tensor palati muscle, which theoretically would be shown by a dragging of the raphé to the non-paralyzed side. Sir Charles Bell, who records many cases of disease of this nerve, does not mention the condition of the palate in any of them. Romberg never saw paralysis of the tensor palati associated with disease of the motor division of fifth nerve; and Dr. Gowers holds the same opinion. Experimental evidence also is not of a satisfactory nature. John Reid failed to elicit palatal movements on irritating the motor division of the trigeminal nerve; and Longet does not mention any effect on the palate when this nerve was stimulated."* Turner placed the motor supply of the tensor veli palatini in the eleventh nerve by way of the otic ganglion. His reasons are entirely theoretical.

In 1862, Chauveau's experiments[8] led him to place the innervation of all of the palatal muscles in the tenth nerve. Rethi,[9] in 1893, wrote that he had torn away the palatal mucous membrane and had observed movements of the tendon of the tensor veli palatini when he stimulated the trigeminal nerve. Vulpian's experiments,[10] however, brought him to the conclusion that the fifth nerve does not supply any of the palatal

* It has already been stated that Reid did not deny the possibility of the relation of the fifth nerve to the tensor veli palatini muscle; and in justice to Longet it should be noted that if one turns to the reference cited by Turner (Anat. et Phys. du Système Nerveux, t. 2, Paris, 1842) it will be found that Longet writes: "La portion non-ganglionaire ou petite racine du trijumeau, galvanisée dans le crâne, détermine des secousses très manifestes dans la mâchoire inférieure, en même temps qu'*elle imprime de légers mouvements au voile palais.* C'est elle, en effect, qui préside au mouvements d'élevation, d'abaissement, de diduction de la mâchoire inférieure, *ainsi qu'à la tension du voile palatin.*" (Italics mine. A. R. R.)

muscles. He placed the motor supply of the palate in the tenth and eleventh nerves, and his experiments suggested the possibility of the ninth as well. In 1905, Cushing [13] described his observations made upon patients in whom the motor root of the trigeminal nerve had been cut during extirpation of the Gasserian ganglion. In one case he stimulated electrically the mandibular nerve at operation and saw movements of the soft palate occur during the stimulation. In four cases, out of a series of twenty, he observed changes in the appearance of the palatal curtain develop after operation, and he attributed these changes to a paralysis of the tensor veli palatini muscle following destruction of the fifth nerve. In the same year, Tissier [14] wrote that the tensor veli palatini is innervated by the seventh nerve.

More recently, Vernet [15] has concluded from an examination of the literature that the fifth nerve must supply the tensor veli palatini muscle; while Davies' studies on the trigeminal nerve [16] led him to write that "the balance of evidence seems to show that the fifth nerve has nothing whatever to do with the nerve supply of the palatal muscles." Among contemporary editions of standard text-books of anatomy we find that Cunningham [16] gives the fifth nerve as the innervation of the tensor veli palatini, while Gray [16] states that the muscle is supplied by a twig from the otic ganglion, and refers to Turner's article which suggests the eleventh nerve as the origin of the ganglionic twig.

EXPERIMENTS

A careful study of the literature made it evident that perhaps the greatest source of error in the attempts to determine the nerve supply of the individual palatal muscles lay in the fact that, almost without exception, previous experimenters and clinical observers have not been able to see the muscles which they were studying. The intact soft palate is watched while a nerve is stimulated; if the palate moves, the nerve is assumed to supply one or more of the palatal muscles; which muscle or muscles it is credited with innervating depends, in each case, upon the imagination of the observer who is attempting to identify the movements of the individual muscles while they lie hidden behind the palatal mucous membrane. Indeed, Hein and Rethi, as far as I have been able to discover, are the only writers who have reported experiments in which the palatal mucous membrane was removed. Hein speaks of stripping the mucous membrane from the belly of the tensor veli palatini muscle in freshly killed animals in order to watch the effect upon the muscle of nerve stimulation; by which he must have meant the nasopharyngeal mucous membrane which he exposed by splitting the head sagittally, since the mere removal of the palatal mucous membrane will not bring the muscle into view. Rethi speaks only of exposing the tendon of the tensor veli palatini, which is not an entirely conclusive experiment, for the reason that the tendon of this muscle fans out into a fascial layer of the soft palate which is closely related to the palatopharyngeus, the pterygopharyngeus and the internal pterygoid muscles as well. The reason why more direct experiments have not been made lies, apparently, in the difficulties encountered in the attempt to expose the palatal muscles in the living animal by dissection, since the irritability of muscle and nerve hardly persists long enough after death to permit a careful dissection of the region and subsequent satisfactory observations. The chief difficulty of the operation seems to have been uncontrollable hemorrhage. Thus, Willems [17] in 1911, after unsuccessful attempts to expose the palatal muscles in living animals in order to study the origin of their motor fibres, wrote: "La moindre incision de la muqueuse est suivie d'une hémorrhage veineuse telle que le champs opératoire en est complètement masqué et que l'animal y succombe en quelques seconds." Such hemorrhage can, however, be controlled perfectly by hemostats and appropriate ligatures, so that the entire operation can be performed with a negligible loss of blood.

The anatomical and physiological relations of the palatal muscles of the dog resemble strikingly those of man; furthermore, the great majority of previous workers have made this animal the subject of their experiments. Dogs were therefore used in all of the present observations. The procedure by which the palatal muscles were exposed with their nerve supplies intact is here quoted from a previous paper: [18]

A dog is anesthetized, tracheotomy performed and connection established with an ether-respiration bottle by means of a tracheal cannula. Both common carotid arteries are ligated to minimize hemorrhage, as it is desirable to introduce about the delicate palatal muscles as few ligatures as possible. The immediate collateral circulation is ample to maintain satisfactorily the nutrition of the structures during the experiment. A mouth-gag is introduced to hold the jaws widely separated, and the tongue is drawn forward and stitched to the lower lip. The hamular processes of the pterygoid bones can be felt on either side as distinct bony prominences in the roof of the mouth. Using these as landmarks, an incision is made through the mucous membrane of the soft palate from a point about one centimeter anterior to them, extending backward in the midline almost to the insertions of the levator veli palatini muscles in the uvular region. Dissection is carried lateralward along the plane of the fibrous layer of the soft palate formed by the tendinous expansions of the tensor veli palatini muscles. The hamular process of the pterygoid bone is exposed, and the palatopharyngeus muscle is seen stretching from this point backward toward the pharynx.* Careful separation of the fibers of this muscle from those of the closely associated pterygopharyngeus reveals the levator veli palatini, which passes between them on its way to the soft palate, forming with them an angle of about 70°. The dissection is continued across the hamular process, and the internal pterygoid muscle is seen just lateral to this landmark, extending anteriorly along the palatine bone. This muscle completely hides the tensor veli palatini in such an approach. It is necessary, therefore, to remove the overlying fibers of the internal pterygoid in order to expose the tensor veli palatini, which can then be seen as a rather pale, very small muscle, the fibers of which extend from the pterygoid hamulus lateralward, backward and upward toward the inferior portion of the temporal bone. The integrity of the nerve supply was, in all cases, tested after completing the dissection by watching for the contraction of the individual muscles during the execution of the swallowing reflex, in which they both normally participate.

*** At this point, ligation of the lateral palatine vessels greatly reduces the liability of hemorrhage.**

Having exposed the tensor and levator veli palatini muscles with their nerve supplies intact, the region is covered with gauze soaked in warm saline. The skull is then trephined and quickly cut away until the brain and medulla are exposed. No attempt is made at this point to arrest hemorrhage, as the animal is immediately decapitated and the brain removed as speedily as possible after cutting the roots of the cranial nerves close to their points of emergence from the brain. The various nerves are then stimulated electrically within the cranial cavity, and the effects of such stimuli are observed by watching the behavior of the naked tensor and levator veli palatini muscles during the stimulation. The irritability of muscle and nerve persists in such a preparation long enough to permit satisfactory observations to be made. For trustworthy results in such experiments, it is necessary to destroy the dural connections between the various nerves, carefully to dry the area about the nerve to be stimulated, and to use a moderate or weak current, preferable with unipolar electrodes. Bipolar electrodes, a strong current and dural or moisture connections between the nerves will, obviously, be productive of unreliable observations because of the diffusion of the current to nerves other than those to which the electrodes are actually applied. It seems certain that some of the incompatible results reported in the literature must have arisen from a neglect of these precautions.

In every experiment, stimulation of the mandibular branch of the fifth nerve caused definite, vigorous contractions of the tensor veli palatini muscle on the side stimulated. The maxillary and ophthalmic divisions of the fifth, and all of the remaining cranial nerves were carefully stimulated in a large number of experiments with no effect whatever upon this muscle. The tensor veli palatini clearly derives its motor supply from the mandibular division of the fifth nerve.

Twigs from the otic ganglion to the tensor veli palatini muscle are commonly described. With the idea of trying to determine its rôle, several attempts were made to paralyze the ganglion. The foramen ovale was enlarged by chipping away the bone about it and the otic ganglion was exposed. The mandibular nerve was stimulated, and contractions of the tensor veli palatini were observed. Then the ganglion was painted with nicotine, and the mandibular nerve was again stimulated. It will be remembered that in all of these experiments fatigue and progressive loss of irritability of muscle and nerve are important factors in the interpretation of observations; but comparison between the experiments in which nicotine was applied and those in which there was no such treatment of the otic ganglion shows no appreciable difference in the time required for loss of response of the muscle to stimulation of the nerve. While this point would bear further investigation, the fact that stimulation of the remaining cranial nerves fails to cause contraction of the tensor veli palatini makes it certain that the muscle does not receive motor fibres by way of the otic ganglion or by any other route from any cranial nerve other than the fifth.

One of the chief objections to the view that the fifth nerve supplies the tensor veli palatini has always been the fact that paralysis of the palate is rarely observed clinically in cases of disease of this nerve. To begin with, it is difficult to understand how one could expect to detect a paralysis of the tensor veli palatini by inspection of the buccal surface of the palate. None of the fibres of this muscle lie in the palate and therefore wasting of the muscle cannot be detected by oral inspection. As for the "theoretical dragging of the raphé to the non-paralyzed side,"[7] this, even if it could occur, would be present only during contraction of the non-paralyzed muscle, and it is to be remembered that the tensor contracts only during certain reflexes (deglutition; yawning; sneezing) and that normally it remains at rest during ordinary elevation of the palate produced by forced respiration or by vowel enunciation." Oral inspection of the palate during swallowing is practically impossible in an unanesthetized patient; and I have found no record of examination of the palate during the yawning reflex in cases of fifth nerve disease. So the fact that palate paralysis has not regularly been observed, does not appear to be a valid objection to the idea that the tensor veli palatini was involved in these cases. But aside from this consideration, a careful study of the action of the tensor veli palatini muscle, with the idea of determining to what extent its contraction influences the condition of the palate, has led to the conclusion that it would be very difficult under any circumstances to detect paralysis of this muscle by inspection of the intact soft palate. Using anesthetized animals, the normal appearance of the palate was observed during deglutition, in which reflex the tensor veli palatini muscles participate. Then, through a small, lateral incision in the mucous membrane just over the hamular process, the tendon of the tensor veli palatini on one side was exposed and cut through. The swallowing reflex was then set in motion again while the soft palate was observed. Although the raphé was marked with India ink in order to be able more easily to detect its movements, there was seen not the slightest dragging of the mid-line of the palate to the non-paralyzed side. The palate was perfectly symmetrical while at rest, and during deglutition the paralyzed side did not present an appearance different in any respect from that of the normal side. After such experiments, a dissection has always been made to make certain that the tendon of the tensor had been entirely severed; and it was further determined that the muscle on the normal side had been functioning properly by watching its contraction during deglutition after it had been exposed by dissection.

If the anatomical structure of the soft palate be studied, it will be found that, after winding around the hamular processes, the tendons of the tensor veli palatini muscles fan out in a fibrous sheet which underlies the nasopharyngeal mucous membrane of the palate. This fibrous sheet has a definite stiffness even when the tensor muscles are at rest; a fact which can be determined by cleanly exposing this layer of the soft palate in an anesthetized animal. If now the tensor veli palatini of one side be stimulated, there will be seen not the slightest deflection of the midline of the sheet toward the side stimulated, even during the most vigorous contractions of the muscle. The tendon will be seen gliding over the hamu-

lar process, but pulling in a direction that proceeds from the margin of the hard palate. It has been pointed out by Henle that the tensor veli palatini is inserted chiefly into the fibrous prolongation of the hard palate, and that its tendinous expansion dwindles away toward the posterior, free end of the soft palate, so that contraction of the muscle cannot affect the soft palate very much. Indeed, Allen wrote in 1883, that at that date it was no longer believed that the tensor veli palatini can actually make the soft palate tense.[19] Debrou [2] and others have recognized this; and one needs no clearer evidence of the difficulty of determining unilateral contraction of the muscle by oral inspection that that afforded by the majority of experimenters who have been unable to see palatal movement on stimulation of the fifth nerve, although the tensor veli palatini was contracting, as the present experiments have shown. Cushing's single case,[12] the experiments of Longet,[7] and two isolated experiments in Hein's series [6] are the only instances which I have found in the literature in which movements of the intact soft palate have been seen on stimulation of the fifth nerve; and it will be remembered that Longet described no dragging of the raphé toward the side stimulated, but only "légers mouvements du voile du palais," [7] while both of Hein's cases were so inconclusive that he resorted to other means in the attempt to establish the relation of the fifth nerve to the palatal muscles. Certainly, contraction of the muscle does stiffen the anterior portion of the soft palate to some degree; but the present experiments indicate that paralysis of the muscle can rarely, if ever, be detected by inspection of the intact soft palate, and then, perhaps, only in cases of unusual development or anomalous insertion of its tendon.

During the progress of these experiments, there was a patient under observation in The Johns Hopkins Hospital who had a parasyphilitic unilateral paralysis in the domain of the third, fifth and seventh cranial nerves. There was a bilateral involvement of the sixth nerve. The remaining nerves were unaffected. There was no palate paralysis, but nasopharyngoscopic examination showed that the pharyngeal orifice of the Eustachian tube on the affected side remained quite stationary during deglutition, offering in appearance a striking contrast to the normal reflex opening of the orifice on the unaffected side. Since it has been shown that the tensor veli palatini is the only muscle which can open the Eustachian tube,[18] this condition was apparently produced by a paralysis of this muscle following disease of the fifth nerve. Such a paralysis of the tensor supports Cushing's suggestion [12] that the aural disturbances occurring after operative destruction of the motor root of the fifth nerve may result from the inability of the Eustachian tube to function properly. It remains for further observation to determine whether such a visually distinct paralysis of the Eustachian tube will be found to be a constant accompaniment of paralysis of the tensor veli palatini muscle.

2. The Motor Supply of the Levator Veli Palatini Muscle

In 1841, Debrou [2] stimulated electrically the various cranial nerves of dogs while observing the effect upon the soft palate, and concluded that the levator veli palatini is supplied by the ninth nerve. Longet's experiments of a similar nature [7] led him to place the motor supply of the levator in the seventh nerve, and the influence of his opinion can be traced through a great mass of clinical and experimental literature concerned with this question. Hein,[6] however, in 1844, wrote that stimulation of the seventh and ninth nerves had no effect whatever upon the soft palate, but that stimulation of either the tenth or the eleventh nerve caused vigorous contractions of the levator veli palatini muscle. Reid [4] wrote, on the basis of his experiments, that the vagus is the principal, if not the sole, motor nerve of the palate. In 1886, Vulpian's experiments [10] led him to the conclusion that the levator veli palatini is supplied by the tenth and eleventh nerves, and perhaps by the ninth as well. Horsley and Beevor,[20] in 1888, described their experiments which led them to place the motor supply of the levator veli palatini in the eleventh nerve. In 1893, Rethi's experiments indicated that the levator is supplied by the tenth nerve. Geronzi,[26] in 1906, added the twelfth to the list of nerves described as innervating this muscle, while Willems,[24] in 1911, wrote that the levator is supplied by the fifth nerve. In 1918, Vernet [13] concluded from an examination of the literature that the evidence seemed to be in favor of the eleventh nerve as the motor supply of the levator veli palatini.

Wolfert [21] pictured his dissections which led him to believe that the levator veli palatini is supplied by a branch formed by the union of fibers from the ninth and tenth nerves, and wrote: "Comparantibus nobis ea, quae in diversis operibus anatomicis et physiologicis de illo musculo ejusque nervis tradita sunt, mirum videbitur, quanta varietas sententiarum de illa re exstet"; and indeed, reference to standard, present-day texts will still arouse in us some wonder, for the nerve supply of the levator veli palatini will be found to be variously given as the tenth nerve;[22, 23] the seventh nerve;[24, 25] the eleventh nerve;[26, 27, 28] and finally, the superior maxillary branch of the fifth.[29]

Experiments

After exposure of the levator veli palatini muscle with its nerve supply intact, in the anesthetized animal, all of the cranial nerves were carefully stimulated within the skull in the manner which has been described above, while the naked muscle was kept under observation. It was at once found that it was impossible to produce contractions of the muscle by stimulation of any cranial nerve other than the ninth, tenth or eleventh. At first, the confusing observation was made that contractions of the muscle would follow stimulation of either of these nerves, although the ninth would cause contractions only occasionally. These three nerves make their exit from the cranial cavity through the same foramen, and as they pass through the foramen they are bound together within the same dural sheath. It is evident, then, that radiation of the current can easily occur when either of the nerves is stimulated. Weak currents were therefore used in a large number of experiments, and it was found that, in all cases, the levator veli palatini would respond to stimulation of the eleventh

nerve; an occasional, inconstant response has followed stimulation of the vagus rootlets, while stimulation of the ninth nerve was entirely without effect.

More definite evidence that the accessory nerve supplies the levator veli palatini was obtained by studying the effect of section of the roots of this nerve in the living animal. In these experiments, after the usual palatal dissection and the determination of the integrity of the nerve supply of both levator muscles, the atlas, the atlanto-occipital membrane and the occipital bone are laid bare by dissection. Following a suggestion of Claude Bernard,[20] a small hole is trephined immediately under the occipital protuberance and a concentrated solution of iron persulphate is injected into the sinus after preliminary ligature of the jugular veins. This procedure coagulates the blood within the sinus, and is a distinct aid in combating the hemorrhage which occurs when the sinus is cut through. The atlanto-occipital membrane is then incised, the dura is entered, and the lower portion of the occipital bone is cut away with rongeurs until the medullary roots of the ninth, tenth and eleventh nerves can be clearly exposed by gently retracting the overhanging cerebellum. Bleeding from the cut surface of the bone is controlled with wax, and great care is taken to try to prevent unnecessary hemorrhage from the small vessels along the cerebellum and medulla, for such bleeding, while unimportant as regards the survival of the animal, tends to collect about and entirely obscure the rootlets of the vago-accessory complex. Having obtained a good exposure of the rootlets of the ninth, tenth and eleventh nerves, the spinal portion of the eleventh on the right side was cut just below the point where it joins the bulbar rootlets. The swallowing reflex was then set in motion, and the levator veli palatini muscles were seen to contract normally on both sides. Then the bulbar rootlets of the eleventh nerve were cut on the right side, after which it was seen that during deglutition, while the levator on the left side contracted vigorously, the muscle on the right side remained motionless. Such an experiment performed upon an animal in whom the soft palate had been left intact produced, during deglutition, the characteristic asymmetry of the palatal curtain which is observed clinically in cases of paralysis of the levator veli palatini muscle. A careful autopsy was performed after every experiment in order to make certain that the rootlets of the ninth and tenth nerves had not been damaged. The evidence obtained from the stimulation experiments, combined with the results following nerve section, lead us to the conclusion that the levator veli palatini muscle receives its motor supply from the bulbar portion of the eleventh nerve.

It is not necessary to assume that experimental error lay at the bottom of the results of the several observers who have concluded that the lower rootlets of the vagus may carry fibres destined for the levator veli palatini. The work of Van Gehuchten,[21] Chase and Ranson [22] and of others has demonstrated that the so-called bulbar portion of the eleventh nerve is, in reality, a part of the vagus, and is entirely distinct, both in origin and distribution, from the spinal portion of the eleventh nerve. In the current edition of Piersol's Anatomy it is stated that "it is generally admitted that the bulbar or accessory portion of the eleventh nerve forms an integral part of the motor division of the vagus and hence should be included with the efferent fibres of the tenth."[23] These facts make it clear that any attempt sharply to limit the functions of the various rootlets must be artificial; and it is possible that in some cases fibres to the levator palati may pass through the lower rootlets of what is commonly regarded as the vagus proper. However, the so-called bulbar rootlets of the eleventh undoubtedly constitute the main route for the motor fibres to this muscle, and I have no evidence that any of the fibres so destined pass through the so-called vagus proper. Even Vulpian, who believed that the inferior rootlets of the tenth (those immediately adjoining the bulbar rootlets of the eleventh) carry motor fibres for the palate, wrote: "Peut-être ces divers filets radiculaires appartiennent-ils tous aux nerfs spinaux ou accessoires de Willis."

SUMMARY

The tensor veli palatini and levator veli palatini muscles have been exposed, with their nerve supplies intact, by dissection in the living animal, and the various cranial nerves have been stimulated within the skull while the naked muscles were observed; and it was determined that:

1. The fifth nerve is the only cranial nerve which supplies motor fibres to the tensor veli palatini muscle. The failure of most clinical observers to detect paralysis of the palate in cases of disease of the fifth nerve arises from the fact that the tensor veli palatini exerts, ordinarily, no effect upon the soft palate that can be detected by oral examination.

2. The method of intracranial stimulation, combined with experimental paralysis of the muscle produced by nerve section, places the motor supply of the levator veli palatini muscle in the so-called bulbar portion of the eleventh nerve (more properly speaking, in the inferior rootlets of the tenth nerve, since the bulbar portion of the eleventh has been shown to be, in reality, an integral part of the vagus).

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ANAPHYLAXIS*

By H. H. Dale

(From the Lister Institute of Preventive Medicine, London, England)

In my first lecture I dealt with the experiments which led us to an explanation of the paradoxical depressor action on the circulation of histamine and similarly acting substances, which are characteristically stimulants of plain muscle. I indicated that this anomalous vasodilator action, which we had been led to regard as an action on the tone of the capillaries, was seen only in certain species, such as the carnivora and the monkey, and showed how, when intensified and prolonged, it led to a shock-like circulatory depletion. Although our attention was confined to the effect on the circulation, there was already perceptible, then, a well-marked divergence between the phenomena seen in different species. This divergence becomes more pronounced when the effects on other organs are brought into the picture, as in studying the general symptoms of poisoning produced by large doses. We have seen already that in the anæsthetized cat such larger doses produce a circulatory shock. In the dog a very similar complex is produced under like conditions. It differs in some details from that seen in the cat. Thus in the dog the preliminary steep fall and secondary rise of blood-pressure are not seen; the arterial pressure falls from the first with the accompaniment of a violent dyspnœa, until it attains a shock-level of some 30 mm. of mercury. The concentration of the arterial blood is quite pronounced, but usually less so than in the cat. The effect in the dog's capillaries seems also to be less generally diffused, those of the liver being more conspicuously affected than others, so that this organ swells and becomes somewhat tense with blood. This is in accordance with the conspicuous liability of the liver capillaries of the dog to the action of poisons of this type, as shown in the experiments of Starling, Thompson, Edmunds and others. On the whole, however, the symptoms in the cat and dog have the same general aspect. In both species hypodermic injection even of large doses produces a prolonged narcosis and lethargy, but is seldom fatal. In the guinea-pig, if histamine is injected into a vein without an anæsthetic, the result is remarkably different. The hair becomes erected, especially on the head, the animal gives a chattering cough, makes violent inspiratory efforts accompanied by convulsive movements of all the limbs, often leaps from the table, falls on its side, and with a few terminal inspiratory spasms is dead. If the chest is opened immediately after death, the heart is found to be beating, while the lungs are fixed in extreme distension, and cannot be made to collapse even by external pressure. A dose of 0.5 mgm. of histamine suffices to kill a large guinea-pig in this manner. If injection be made intraperitoneally much larger doses fail to kill, and the condition produced is that of a prolonged collapse, with pronounced respiratory embarrassment; recovery may take place even after hours. During the collapse a conspicuous feature of the condition is the rapid fall of body temperature. We have observed a fall in the rectal temperature from 38° to 28° C. in an hour, with subsequent recovery, after intraperitoneal injection of 3 mgm. of histamine.

In the rabbit, again, with hypodermic or intraperitoneal injection comparatively large doses are tolerated, with the narcotic effect which seems common to all species. Intravenously, much smaller doses cause rapid death with convulsions, the actual cause of death being, in this case, an acute failure of the right chambers of the heart, the respiratory center continuing to act for a brief period after the heart has stopped. This acutely fatal effect in the rabbit, though its mechanism is different, resembles the asphyxial death in the guinea-pig in being prevented or modified by deep anæsthesia. If the rabbit is deeply under urethane this fatal effect on the heart does not appear; under such conditions, as I have mentioned earlier, histamine produces a simple rise of the arterial pressure.

These effects in the different species, superficially so dissimilar, are not difficult to understand in the light of the analysis of the effects of histamine, with which I dealt in my last lecture. You will remember that its different effects could

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be interpreted as due to its contrasted actions on plain muscle and on the walls of the capillaries, the former being stimulated to tonus, while the normal tone of the latter is weakened or abolished. In the carnivora this effect on the capillaries, leading to peripheral accumulation of blood and depletion of the heart, is the predominant factor in the effect; and not only is it not suppressed, it is even intensified by prolonged anæsthesia with anæsthetics of the alcohol series. In the guinea-pig and the rabbit the effect on plain muscle comes into the foreground. In the guinea-pig the plain muscular coats of the bronchioles are so richly developed and reactive, the tubes are so small and their lining mucous membrane so thick and folded, that, as Schultz and Jordan have shown, there is easily produced a valve-like obstruction of the air-way, leading to asphyxia, with extreme, fixed distension of the lungs. In the rabbit the failure of the right side of the heart seems to be due mainly to intense constriction of the branches of the pulmonary artery, causing the right chambers of the heart to become overdistended, so that they pass into a fatal fibrillation. Probably the effect is accentuated by some measure of bronchial constriction, but this is not, as in the guinea-pig, the predominant factor in the result. Deep anæsthesia with ether or urethane greatly weakens in both species these plain muscle effects, whether on the bronchi or on the pulmonary arteries.

I have devoted more time to the analysis of these effects, with their curious appearance of contrast in various species, than the subject would probably deserve, if we were concerned merely with the action of histamine as a pharmacological curiosity. Their real interest appears when we find the same types of action, with the same contrasts between the different species, appearing not only in the reaction of the different animals to a large class of protein poisons, but also in the reaction of the same types, when specifically sensitized, to normally inert proteins, in the so-called anaphylactic shock. No one familiar with the anaphylactic shock, as seen in different species, can fail to be struck with the extraordinary similarity. Here, again, the conspicuous feature of the effect in the anæsthetized dog is the profound collapse of the systemic arterial pressure, as Biedl and Kraus, Manwaring, Pearce and Eisenbrey, among others, have shown. Here, too, in the guinea-pig, especially when the sensitizing protein is reinjected intravenously, the picture is dominated by an intense bronchial spasm, as first shown by Auer and Lewis, producing an asphyxiation so rapidly and dramatically fatal that other effects usually have not time for development. In the rabbit, again, the main feature of the anaphylactic shock is heart-failure, as Auer demonstrated.

In some respects the anaphylactic shock differs from that produced by histamine, and resembles more closely that produced by more complex proteinogenous poisons, such as Witte's peptone. For example, the loss of the normal property of coagulation of the blood, especially in the dog, is characteristic of the anaphylactic shock, and of the action of Witte's peptone, but not of that of histamine. In the dog, too, the concentration of the capillary poisoning on the vessels of the liver is more conspicuous in the anaphylactic shock and in the action of peptone than in that of histamine. Manwaring showed, indeed, that in the dog exclusion of the liver from the circulation wholly, or almost wholly, abolished the circulatory features of the anaphylactic reaction. This was confirmed by Voegtlin and by Bernheim, by a more elegant method. In the rabbit, heart failure again seems to be a common feature in the different actions. Till recently it appeared doubtful whether the mechanism of its causation in the anaphylactic shock is identical with that which I have described in the case of histamine. Auer attributed the failure of the rabbit's heart in anaphylactic shock to a poisoning of the heart muscle; but quite recently Coca has reinvestigated the effect, and has demonstrated the predominant share in its production of an intense constriction of the pulmonary arterioles. The resemblance to the effect of histamine is, therefore, established in this case also. It is evident that we may safely regard the anaphylactic symptoms in the dog, the guinea-pig and the rabbit—in which species they have most thoroughly been studied, as due to a complex of contraction of plain muscle and loss of capillary tone closely resembling that produced by histamine; and the appearance of this syndrome again in the reaction of a specifically sensitized animal to a normally inactive protein gives abundant food for reflexion. The similarity is so striking that it is impossible to avoid the suggestion of a community of cause, leading to a community of effect. It is to a consideration of the meaning of this correspondence that I wish to devote the greater part of this lecture.

We must, in the first place, review as rapidly as possible what is known as to the nature of the anaphylactic condition, and of the events which lead to the appearance of the symptoms known as the anaphylactic shock. Knowledge has already moved far from the position in which anaphylaxis was regarded as the opposite of immunity. You will remember that, when Richet first clearly recognized the condition, the accidental circumstances of his experiments gave the phenomenon this appearance of a mere accentuation of sensitiveness to certain protein poisons. He happened to be working with poisonous proteins from sea-anemones, from edible mussels and from a poisonous plant—*Hura crepitans*. These proteins had of themselves, on a normal dog, a poisonous action of the general type which we have been discussing. When a dog, which had received a sub-toxic injection of one of them, was given a second injection of the same poison some weeks later, it responded with a violent, immediate and often fatal reaction, which was easily mistaken for a response of the kind given by a normal dog to a large dose, but accelerated in its onset and aggravated in its severity. Richet supposed that he had found the converse or antithesis of immunity, and the legacy from that mistake is the name "anaphylaxis." This has nothing to be said in its favor from the point of view of etymology; it gives faulty expression to a mistaken conception; but usage has established it firmly.

The true nature of the phenomenon soon became apparent. Arthurs demonstrated the increasingly poisonous action on the rabbit of a second and subsequent injections of a substance normally so innocuous as horse serum. It was made clear that

the symptoms resulting from the second injection of a protein, when this followed the first injection at a suitable interval, had no connection with the normal toxicity of the substance. An animal, indeed, might be and often was at the same time immune to the normal toxic action of a protein, and anaphylactic to it simply as protein. Anaphylaxis was a condition in which the animal reacted to a normally harmless substance as to an acute poison.

The recognition by Theobald Smith and others in this country of the extreme sensitiveness to horse-serum, shown by guinea-pigs which had survived a standardizing test for antitoxin, constituted practically a rediscovery of anaphylaxis in another species, and gave an immense impetus to its investigation. Thenceforward, the special characteristics of the guinea-pig have made it the chosen subject of a large proportion of the experiments dealing with the phenomenon. The ease and regularity with which animals of this species can be sensitized, the minuteness of the dose which suffices for their sensitization, and the rapidly lethal character of their response to reinjection, have greatly impressed the imagination of investigators, and have given almost a sensational interest to the phenomena.

There has been a perceptible danger, in some quarters, lest the somewhat dramatic symptom-complex shown by the guinea-pig might be itself regarded as characteristic of the anaphylactic reaction. It is necessary to keep clearly in view that the characteristic of the anaphylactic condition is not the nature of the symptoms, which a large number of protein poisons evoke, but their appearance in response to the injection of a substance which normally has not this action, owing to a specific sensitiveness conferred by a previous injection. Bearing this in mind, and also being careful not to generalize too hastily from results obtained only on the guinea-pig, we may consider the evidence as to the nature of anaphylaxis, obtained chiefly by experiments on that species.

The main features of the condition were soon made clear. A minimum interval of 6 to 10 days, according to the size of the dose and the method of giving the reinjection, must elapse before the abnormal sensitiveness could be detected. It then rapidly attained a maximum development, and in the guinea-pig it lasted for the rest of the natural life of the individual. The specific sensitiveness could be transferred to a normal guinea-pig by injection of blood or serum from a guinea-pig, or other animal, which had been rendered anaphylactic; so that there was a "passive" anaphylaxis, corresponding to a "passive" immunity, and indicating that anaphylaxis, like immunity, depends on the presence of a specific antibody of some kind. But there is one curious feature in the production of this passive anaphylaxis in the guinea-pig, which is too frequently left out of account in discussing the nature of the condition. The passive anaphylaxis does not appear immediately, even if the anaphylactic serum is injected intravenously. The first traces of sensitiveness appear only after the lapse of some 5 or 6 hours, and the condition is not fully developed for 24 hours. While, therefore, everything points to the dependence of the anaphylactic condition on the presence of a specific antibody, which can be transferred from one animal to another, the mere presence of this antibody in the blood is not sufficient to produce the condition. Time must be allowed for its development, and we are driven to suppose that the antibody must acquire some special relation to the constituents of the body fluids or the tissues, before it can become effective in rendering the animal anaphylactic.

The anaphylactic state appeared to be so unlike those usually associated with the presence of antibodies, that it was at first naturally supposed that the anaphylactic antibody was of a special type. It must still be recognized as possible that it may be so. On the other hand, there is a great deal of evidence which points in the direction of its identity with the so-called "precipitin"; that is to say, with an antibody having this property, that, when it is mixed with the antigen, the two undergo a mutual aggregation of their colloidal particles.

The evidence for this identity is mostly indirect, but none the less highly suggestive. The type of substance which is preeminently effective in producing anaphylaxis is that which characteristically causes precipitin formation, namely, that of the large-moleculed native proteins. Modifications which destroy the power of evoking formation of precipitins, or change the specificity of the protein with respect to this reaction, have a similar effect on its character as an anaphylactic antigen.

There is, again, a suggestive similarity between the limits of the specific discrimination shown by the two types of reaction. Both discriminate clearly between analogous substances, as for example the respective blood sera, from different species; but in neither case is the distinction absolute. In both cases there is a blurring of the distinction, an overlap of the specificity, when dealing with sera from related species; and the higher the degree of sensitiveness of the animal, or the more intense the precipitating quality of the immune serum, the greater the extent of the non-specific reaction. An animal which has received a previous injection of horse-serum acquires a high degree of sensitiveness to that serum, and usually shows no abnormal reaction to similar doses of ox or sheep serum; but when the sensitiveness to horse serum is very pronounced, the animal may react in a minor degree to large doses of serum from the ox or sheep.

Not only substances from different species, but the constituents of different organs, and even different individual proteins from the same organ, behave as separate antigens, distinguishable either by the anaphylactic or the precipitin reaction, though the discrimination is, again, not absolute in the case of either reaction. Wells was able to distinguish separate antigenic properties by the anaphylactic reaction in five different proteins from the hen's egg. Hartley and I worked with the three separate pure proteins from horse-serum, euglobulin, pseudoglobulin and albumin and found that they were similarly distinguished by the anaphylactic reaction in the guinea-pig, the distinction being very similar to that found by Andrew Hunter by means of the precipitin reaction. Still more suggestive of an identity of the anaphylactic antibody and the precipitin is the observation that serum from a rabbit, in which a strong precipitating reaction for a certain protein

has been produced, if injected into a normal guinea-pig, will render the latter anaphylactic to the same protein; and that the more powerful the precipitating quality of the rabbit's serum, the smaller the dose needed to produce a given degree of the specific sensitiveness in the guinea-pig. Strongly, however, as all such comparisons suggest an identity or close association between the anaphylactic antibody and the precipitin, it is not possible to regard anaphylaxis as due merely to the presence of precipitin in the blood. The serum from an anaphylactic guinea-pig forms no visible precipitate with the sensitizing antigen in any dilution. It is difficult, indeed, in the guinea-pig, so easily and regularly rendered anaphylactic, to obtain a precipitating serum; whereas the rabbit, which so readily yields a precipitating serum, is sensitized with relative difficulty and irregularity. By repeated injections, at intervals too short to allow the development of anaphylaxis, a precipitating quality may be induced in the guinea-pig's serum; but the animal is then not anaphylactic, but *immune*.

This, sketched with necessary brevity and in barest outline, omitting, perforce, details of the enormous mass of published experiments or reference to their authors, is the essence of the problem of the anaphylactic condition, as it presents itself to me. The two points which I ask you to bear in mind are (1) that anaphylaxis is a condition in which a normally innocuous, indeed, an inert substance has the effect of an acute poison of the type whose action we endeavored to analyze yesterday; (2) that the condition is apparently dependent on the formation or introduction of a specific antibody of the precipitin type; but that, when the abnormal sensitiveness is most clearly and characteristically developed, no precipitating quality is recognizable in the blood serum.

There are two lines along which these puzzling and seemingly contradictory indications can be explained. Both have been followed, by different writers and investigators. One of the earliest conceptions of the difference between anaphylaxis and immunity, put forward by Besredka and at one stage adopted by Friedberger, supposed that in the anaphylactic animal the antibody was fixed to the cells of the tissues, so that its interaction with the antigen occurred in the cells, whereas a sufficient excess of antibody in the circulating fluids fixed the antigen before it could reach the tissues, and thus produced immunity. Such a conception gives an intelligible explanation of the relation and difference between anaphylaxis and immunity. I still believe it to be fundamentally true, and propose presently to put before you evidence which seems to me to speak very strongly in its favor. It is incomplete, however, in that it does not immediately provide an explanation for the very remarkable resemblance between the anaphylactic shock and the action of naturally poisonous protein derivatives.

This resemblance formed the starting point for another type of theory. I believe Professor Vaughan was, at any rate, one of the earliest observers to put forward the idea that the anaphylactic condition depends on the production of a highly specific ferment, capable of hydrolysing the particular protein to which the animal had been rendered sensitive, and no other. On this view, when the sensitizing protein is reinjected, the specific ferment immediately begins to split it, liberating toxic cleavage products, and so causing the shock. In this form the theory is not likely, I think, to win permanent acceptance. It shares with Abderhalden's conception of the "Abwehrfermente" the defect that it postulates the existence of ferments having a more specific relation to a protein substrate than any known ferment has been found to possess. A ferment which will hydrolyse horse protein, but not attack sheep protein is something quite different from ferments as we know them. Mere quantitative considerations also make it very difficult to accept the view that the anaphylactic shock is due to such fermentative cleavage of the antigen itself in the blood-stream. The dose of a pure protein, which suffices to kill a guinea-pig rendered anaphylactic to it, is much smaller than the fatal dose of the most powerful of the known poisonous protein derivatives producing this type of action. One twentieth of a milligram of pure egg albumen will kill a guinea-pig rendered sensitive to it; it requires about 10 times that dose of histamine, the most powerful of the known poisons of this class. Different attempts have been made to bring this theory of the fermentative production of a poison in the blood into harmony with the specificity of the anaphylactic reaction, and its apparent relation to the precipitin phenomenon. Friedberger supposed that the complex of antigen and antibody was formed in the blood, and there condensed upon itself a proteolytic ferment, somewhat gratuitously identified with the so-called "complement." He supposed that if the precipitating antibody were present in sufficient excess, hydrolysis took place so quickly as to overshoot the toxic stage, so that the animal was rendered immune.

Summarized thus baldly, but I think not unfairly, the theory seems somewhat fantastic, but it had a large influence on the course of the investigations devoted to this problem for several years. Its experimental basis was slight. Friedberger collected the specific precipitate, formed by the mixture of precipitating serum from a rabbit with its corresponding antigen, and digested this with fresh serum from normal guinea-pigs. The serum became toxic, and in some cases a dose of 4 or 5 c. c. of such treated serum caused death in guinea-pigs, with symptoms of the type supposed to be characteristic of the anaphylactic reaction. It was soon found, however, that "anaphylatoxin," as such toxic guinea-pig serum came to be called, could be prepared in many ways. If the serum was digested with suspensions of bacteria, or even with suspensions of kaolin or kieselguhr, it acquired a similar toxicity. Bordet found that digestion of guinea-pig serum with a small proportion of a sol of agar-agar would impart to it the characteristic toxicity, and Sachs and Nathan obtained similar effects by digesting it with starch or inulin. These phenomena of the so-called "anaphylatoxin formation" have attracted a very large share of the investigation devoted to anaphylaxis. Theories as to their meaning at the present time are of two chief kinds. In the first place Jobling and his co-workers, and also Bronfenbrenner, have published inter-

esting and highly suggestive series of studies on the relation between the natural tryptic ferment, present in all serum, and the inhibitory factors, belonging to the so-called "antitrypsin," which normally keep its activity in abeyance. They have described a number of physical or chemical agents which destroy or weaken this antitryptic power of normal serum, liberating the activity of its tryptic ferment and initiating a self-digestion of the serum proteins. Such autodigestion renders the serum toxic, and it is supposed by this school of investigators that the formation of the complex of antigen and antibody, when the former is reinjected into an anaphylactic animal, has the function of removing antitrypsin from the sphere of action, thereby initiating autodigestion and the production of toxic cleavage products from the animal's own blood proteins. Such a conception successfully evades the quantitative and other difficulties attaching to the theory of a specific ferment digesting the antigen. It apparently fails, however, to account even for some of the most characteristic examples of anaphylatoxin formation *in vitro*. Incubation of serum with agar or starch does not bring about any perceptible hydrolytic cleavage of the proteins; but such serum, injected into a guinea-pig, reproduces the symptoms seen in the anaphylactic shock with far greater fidelity than serum in which recognizable autolysis has been induced by other forms of treatment. Another view has, therefore, gained adherents, which attributes the toxic action of serum treated with various colloids to some rather vaguely defined disturbance of the colloidal equilibrium of its proteins. Similarly the anaphylactic shock is attributed to a disturbance of the equilibrium of the colloids of the blood, brought about by the union in it of the antigen and antibody.

I make no pretence to do justice, in the limits at my disposal, to the mass of ingenious and careful experiments devoted to this phenomenon of anaphylatoxin formation. I attempt no judicial summary. I can only try to indicate to you the impression it makes on one who has devoted some thought and personal investigation to this problem of anaphylaxis. I believe that these observations on the production of toxic sera *in vitro* will in time find their place in the true conception of the anaphylactic reaction, but that their bearing on its meaning will not be of such a direct and obvious kind as has been widely assumed. I think an indication of the true position can be detected in the recent publications of Novy and de Kraif. It has long been known that blood, in clotting, acquires a toxic action of the general type to which I have so often alluded. In the serum, as it separates from the clot, this toxic action rapidly declines, but by no means wholly disappears. It can be revived and enhanced by various forms of treatment with absorbent suspensions or sols of colloidal substances. The weak point of the evidence, as a contribution to the theory of anaphylaxis, is the lack of clear connection between this artificial production of toxic serum and the anaphylactic reaction as seen in the living animal. If the production of "anaphylatoxin" *in vitro* really represented the process occurring in the circulation and causing the anaphylactic shock, the substances having this action *in vitro* should be even more effective when injected into the living circulation; and, on the other hand, the most effective combination for producing anaphylatoxin outside the body should be that of serum from an anaphylactic animal with its corresponding antigen. In neither case, however, is this expectation fulfilled. Sols of agar and starch, which so readily and regularly make serum toxic, can usually be injected into the living blood-stream with impunity; and though Richet, Vaughan, Novy and some others have on occasion succeeded in producing a toxic mixture by incubating anaphylactic serum with antigen, such successes have been but rare exceptions among the predominant failures recorded by those who have tried this simple experiment. It can hardly be doubted that such an obvious and directly significant method of preparing "anaphylatoxin" would have been the usual one, if it gave any reasonable chance of success.

It seems to me, further, that the interest aroused by this work on the production of toxic sera has diverted attention from some of the essential features of the problem presented by anaphylaxis. Such experiments throw no real light on the difference between anaphylaxis and immunity; they do not touch on the characteristic and significant feature of passive anaphylaxis in the guinea-pig, namely, the fact that the introduction of the antibody into the circulation of a normal animal does not immediately render it sensitive to the corresponding antigen, but only after an interval of some hours. I know of no conception which throws any light on these essential features of the problem except that which relates the anaphylactic condition to a location of the antibody in the cells rather than in the blood-stream, and attributes immunity, on the other hand, to an excess of the antibody circulating in the blood. To the evidence in favor of this conception we may now return.

The evidence has been obtained along two lines. One was followed with great ingenuity and striking success by the late Richard Weil, by whose death during the war your country and this enquiry have lost an enthusiastic and brilliant investigator. Weil studied the phenomena of passive anaphylaxis, as produced in the guinea-pig by injecting a highly precipitating serum from a rabbit. He found, as others had done, that the sensitiveness did not appear immediately after the injection, even when this was given intravenously, but needed about 24 hours for its maximum development. By bleeding guinea-pigs at intervals after the injection, and injecting this blood into yet others, he was able to follow the fate of the antibody in the blood, and its relation to the development of sensitiveness. He found, in general terms, that sensitiveness only appeared as the antibody disappeared from the blood, and that only a small amount of antibody was left in circulation by the time the sensitiveness was fully developed. He found, further, that when the animal had thus become sensitive, a further injection of a large dose of the antibody had a protective action, rendering the animal temporarily immune. So far, then, the evidence obtained along these lines was completely in accord with the conception of anaphylaxis as due to predominant location of the antibody in the tissues, and of immunity as due to its presence in excess in the blood.

At about the same time this question of the location of the antibody was being attacked by an entirely different method. You will remember that in the guinea-pig the characteristic and dominating feature of the anaphylactic reaction is the contraction of the plain muscle of the bronchioles, causing an asphyxiating obstruction of the air-way. If this were caused by a reaction taking place in the muscle cells, without the intervention of the blood, the effect should be obtained with the plain muscle isolated from the body, and freed as far as possible from traces of blood. This was first attempted by Schultz, in Washington, who demonstrated the fundamental fact of the reaction of isolated intestinal plain muscle from

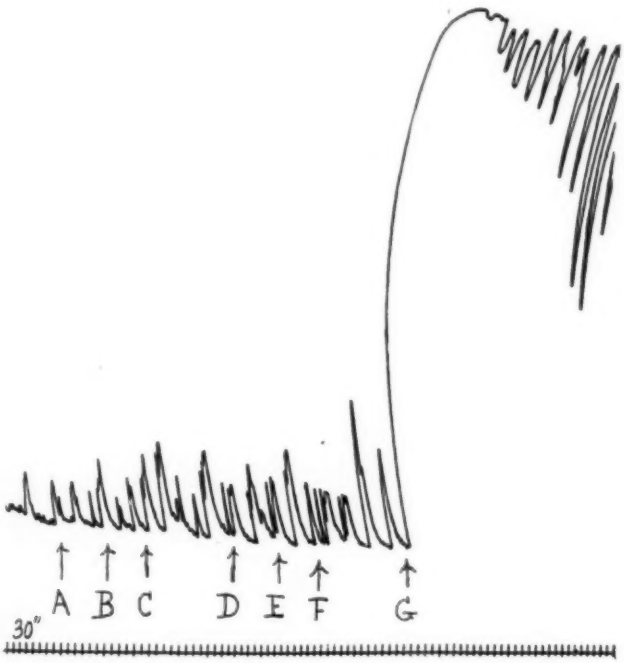


FIG. 1.—Sensitization: 1/400 c. c. antitoxin (horse)+1 test dose of toxin, 14 days. Doses in each case 0.1 c. c. *A*, sheep. *B*, cat. *C*, rabbit. *D*, dog. *E*, human serum. *F*, egg-white. *G*, horse serum. (Jour. of Pharm. and Exp. Therap., Vol. IV, p. 214, Fig. 26.)

the anaphylactic guinea-pig, when the specific antigen was added to the bath of Ringer's solution in which the muscle was suspended.

There were features of the method used by Schultz which made it somewhat difficult to disentangle the specific reaction to the antigen from the effects on normal isolated plain muscle of native sera, especially when fresh. My own attention had meanwhile been drawn accidentally to the same phenomenon. In connection with the work on histamine I was examining the action of a number of organ extracts and body fluids on the isolated uterine muscle of the guinea-pig. Certain preparations exhibited an astounding sensitiveness to horse serum, and enquiry elicited the fact that these were from guinea-pigs which had survived an antitoxin-standardization. As soon as I had the opportunity, I made a deliberate study of this phenomenon, and found that it was possible to demonstrate all the characteristic phenomena of anaphylaxis on the isolated plain muscle *in vitro*. The sensitiveness exhibited was of great intensity, and highly specific; the reaction was obtained as well with highly purified proteins, devoid of action on the normal plain muscle, as with fresh sera; the plain muscle which had once been effectively stimulated by its specific antigen, had lost its natural sensitiveness, but could be resensitized *in vitro*. In fact, all the recognized phenomena of the anaphylactic condition could be demonstrated as easily on the isolated muscle, washed by prolonged perfusion from all removable traces of blood, as in the whole animal.

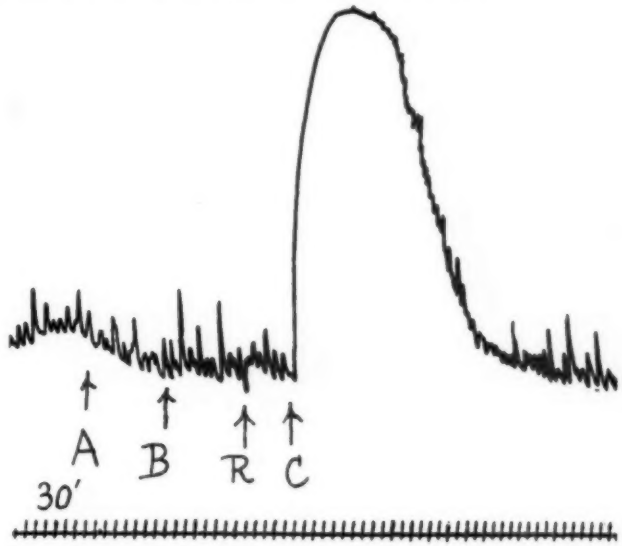


FIG. 2.—Sensitization: 1/540 diphtheria antitoxin (horse)+1 test dose of toxin, 14 days previously. Perfused. Bath volume 250 c. c. At *A* added 0.5 c. c. sheep serum. At *B* added 0.5 c. c. cat serum. At *R* (in this and all other figures) run off the fluid in the bath, wash out, and replace by clean Ringer's solution. At *C* added 0.1 c. c. horse serum. (Jour. of Pharm. and Exp. Therap., Vol. IV, p. 177, Fig. 1.)

The figures * will indicate or recall the nature of the evidence obtained by this method.

Fig. 1 illustrates the close specificity of the reaction. A horn of the uterus from a guinea-pig which had received, fourteen days previously 0.0025 c. c. of horse serum was suspended in a bath containing 250 c. c. of Ringer's solution. At *A*, *B*, *C*, *D*, *E* and *F* were added doses of 0.1 c. c. of each of the following: sheep, cat, rabbit, dog and human serum, and egg-white. None produced any change in the low tonus and small natural rhythm exhibited by the muscle. At *G* 0.1 c. c. of horse serum was added, and the plain muscle promptly responded with maximal tonus.

Fig. 2 shows the reaction of plain muscle from a similarly sensitized guinea-pig. The uterus was, in this instance, perfused for an hour with warm Ringer's solution before removal from the body for experiment. It gives no response to 0.5 c. c. of sheep serum, added at *A*, or to 0.5 c. c. of cat serum at *B*,

* Shown in the lecture as lantern slides.

but gives the characteristic maximal tonus in response to 0.1 c. c. of horse serum, added at *C*.

Fig. 3 shows a record on a faster moving surface: the response to the specific antigen, added at *A*, is seen to begin within 10 seconds—a latent period as brief as that observed with the action of many stimulant drugs on the normal plain muscle.

FIG. 3.—Sensitization: 1/480 diphtheria antitoxin + 1 test dose toxin, 14 days. Not perfused. Bath volume 250 c. c. Tracing taken with faster drum than that used in Fig. 2, to measure rapidity of onset of effect. *A*, 0.5 c. c. horse serum. (Jour. of Pharm. and Exp. Therap., Vol. IV, p. 178, Fig. 2.)

Figs. 4 and 5 illustrate several points. In the first place, the plain muscle is from a guinea-pig rendered *passively* anaphylactic to horse serum. The muscle has been thoroughly washed by perfusion, but still shows the characteristic response to 0.05 c. c. of horse serum, added at *A* to the 20 c. c. of Ringer's solution in which the muscle is suspended. After this effective stimulation, and a change to fresh Ringer's solution at *R*, the muscle is completely insensitive, responding as little as normal plain muscle to a second similar dose of horse serum added at *B*. After being washed again the muscle was soaked for 2½ hours in a 10 per cent dilution of serum from guinea-pigs anaphylactic to horse serum, and then washed in numerous changes of clean Ringer's solution. The response to a further 0.05 c. c. of horse serum at Fig. 5, *E* shows that

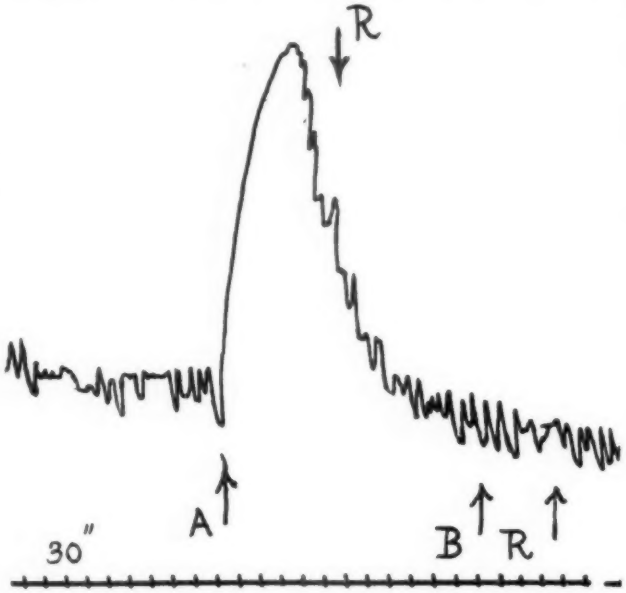


FIG. 4.—Passive sensitization with serum from guinea-pigs anaphylactic to horse serum (see test). Perfused. Bath volume 20 c. c. *A*, 0.05 c. c. horse serum. *B*, the same. (Journ. of Pharm. and Exp. Therap., Vol. IV, p. 198, Fig. 15.)

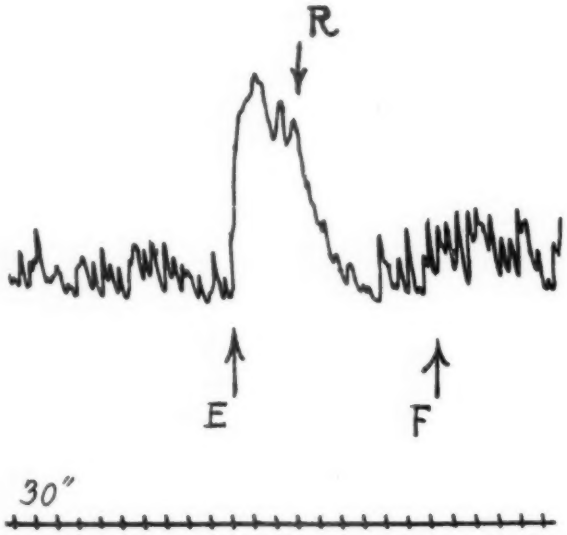


FIG. 5.—Same experiment as in Fig. 4. After resensitization *in vitro* by soaking for 2½ hours in 10 per cent serum from horse sensitive guinea-pigs. *E*, 0.05 c. c. horse serum. *F*, the same. (Journ. of Pharm. and Exp. Therap., Vol. IV, p. 199, Fig. 16.)

passive sensitiveness has been reconferred upon the muscle *in vitro*, and again removed by an effective dose of the antigen, since another dose of horse serum at *F* is without effect.

Other slides, not here reproduced, showed that the surviving plain muscle from a normal guinea-pig could be rendered

anaphylactic by prolonged perfusion with diluted serum from anaphylactic (or immunized) guinea-pigs. Such "passive sensitization *in vitro*" was hardly perceptible after one hour's perfusion, but was definitely in evidence after five hours' perfusion; a clear analogy to the first appearance of passive anaphylaxis *in vivo* four to six hours after intravenous injection of the anaphylactic or immune serum.

Fig. 6 illustrates the response of plain muscle from a guinea-pig rendered *immune* to horse serum by a series of suitably spaced injections of increasing size. Control guinea-pigs, treated in parallel with this one, received on the day of this experiment 5 c. c. of horse serum intraperitoneally, without showing symptoms of intoxication. It may safely be assumed, therefore, that the guinea-pig from which the uterus was taken

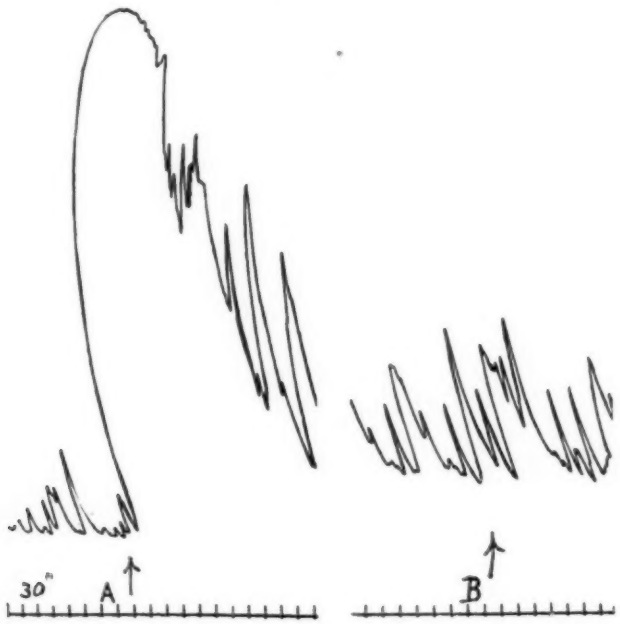


FIG. 6.—Guinea-pig immunized to horse serum. *A*, 0.5 c. c. horse serum. *B* (after change), 0.5 c. c. sheep serum. (Journ. of Pharm. and Exp. Therap., Vol. IV, p. 194, Fig. 13.)

was also immune. Nevertheless the plain muscle, washed carefully from blood by thorough perfusion, is found to be as highly sensitive to horse-serum, of which 0.5 c. c. was added at *A*, as that from many anaphylactic animals.

Since the serum from such immune guinea-pigs is much more effective in conferring passive anaphylaxis, on normal guinea-pigs, than a serum from those which are themselves actively anaphylactic, the conclusion seems to be inevitable that they owe their immunity to the presence in the blood of such an excess of antibody, that the antigen is fixed before it reaches the sensitive tissue cells. The sensitiveness of these can only be revealed, when they are washed clear from the protecting blood, as in this experiment, and brought into contact with the antigen *in vitro*.

The experiments make it clear that there are two ways in which the animal may lose a specific sensitiveness once acquired. An effective, but not fatal, dose of the antigen will temporarily free the tissue cells from antibody, so that, until sensitiveness is reacquired, they have regained their normal indifference to the antigen; on the other hand, if injections be continued at suitable intervals, a condition is reached in which, though the tissues are sensitive, they are protected by excess of circulating antibody. These entirely different conditions, the temporary "desensitization" and the much more lasting true "immunity" have been widely confused in the literature by the application to both of the awkward and misleading term "antianaphylaxis."

These observations on isolated plain muscle, which have been abundantly confirmed by others, seem to leave no escape from the conclusion that the specific sensitization of the guinea-pig's tissue cells, by the presence in them of the antibody, is a reality, and that a true anaphylactic reaction is obtainable without the intervention of the blood at all. The same conclusion is indicated by the work of Coca, who showed that practically the whole blood of an anaphylactic guinea-pig could be replaced by blood from normal guinea-pigs without materially affecting the animal's reaction to the sensitizing antigen. Rumpf also, by an ingenious method of measuring the metabolic activity of the liver cells, showed that these cells in the anaphylactic guinea-pig were directly poisoned by the antigen, irrespective of the presence of its own blood.

I think it may be taken as almost generally accepted that anaphylaxis in the guinea-pig is largely the result of this location of the antibody in the tissue cells, though there is some evidence, provided, for instance, by recent experiments of Manwaring, for believing that the presence of the blood, containing some antibody, is a factor in the shock. Indeed, since there must be an equilibrium of distribution of antibody between the blood and the tissue cells, it is not reasonable to suppose that the latter will retain their full complement of antibody if the blood is washed away. In other species the position is less clear. Manwaring, and later Voegtlin and Bernheim showed that in the dog the characteristic shock does not appear when the liver is excluded from the circulation. It has usually been supposed that this indicates an elaboration of toxic substances in the liver as the cause of the shock, but Weil's experiments on the localized effect of antigen injected into the blood going only to one lobe of the liver, seems to indicate that here also we are dealing with a case of cellular sensitization.

In the rabbit, on the other hand, the evidence as yet available is predominantly in favor of the shock being due to an interaction of antigen and antibody in the blood. This animal, however, occupies in many respects an anomalous position. It is unique in the readiness with which the presence of precipitating antibody in its blood can be evoked; it is rendered sensitive with relative difficulty and uncertainty; it shows no clearly marked distinction between the conditions of anaphylaxis and immunity; it cannot be desensitized by a non-fatal reinjection, or rendered passively sensitive.

Let us confine our attention, for the moment at the least, to the phenomena in the guinea-pig, in which animal they

have been thoroughly studied, and in which their meaning is relatively clear. We have arrived at the conception of anaphylaxis in this species as due to the interaction of antigen and antibody occurring in the cells. We are still faced, however, by our initial problem. We have still to ask why the occurrence of this interaction in the cells should produce effects on plain muscle and capillary endothelium closely resembling those produced by a group of protein-derivatives, of which we took histamine as a type. The resemblance is too striking to be merely accidental; there must be some community of cause determining the community of result.

We are venturing now beyond the region of experimental observation into that of speculation, and there is abundant room for difference of opinion. It is largely a matter of balancing probabilities, or of choosing that conception which involves the smallest number of assumptions, or which is most fruitful of suggestion for further experiment. In endeavoring to find a common factor we may proceed in either direction; we may endeavor to interpret the anaphylactic reaction in terms of what we know of the action of poisonous protein derivatives, or we may endeavor to interpret the action of these normally poisonous substances in the light of what we know of the nature of the anaphylactic reaction. In a general way these alternatives correspond to the two types of theory I have already mentioned, which I may call the "proteolytic" and the "physical" theories of the anaphylactic shock. Let us consider the former, which attributes the resemblance between the anaphylactic shock and the effects of poisonous protein derivatives to an actual production of such derivatives in the anaphylactic reaction, the union of antigen and antibody being supposed in some way to determine the onset of a protein cleavage. In the light of the further evidence we have considered, this process must be transferred from the blood to the functionally vital cells, and this transfer makes the theory less difficult to accept. The supposition that the poison is produced in close contact with the reacting structures, largely if not wholly, removes, as Professor Abel has recently pointed out, the difficulty of harmonizing this theory of the fermentative production of a poison with the practically immediate onset of the symptoms, or of the response of the isolated tissue. I think, further, that there can be little doubt that the union of antibody and antigen *is* actually the prelude to a proteolysis. There is good evidence that the reinjection of a foreign protein, into an animal which has had previous injections of the same, is followed by an additional excretion of nitrogen in the urine, much in excess of that which could be derived from the antigen. On the other hand, the magnitude of this excess of nitrogenous output, representing excess of protein breakdown bears no kind of relation to the severity of the symptoms caused by the injection; it is greater in the immune animal, which shows no symptoms, than in the anaphylactic. I think, therefore, that the most that can be said for the proteolytic theory is that, if the injection of the antigen into the anaphylactic animal were to cause the liberation of protein cleavage products, or even of histamine, as Professor Abel seems to think not improbable, within the active cells of the body, the result might well be such a reaction as we see in the anaphylactic shock.

The other, which I may call the physical hypothesis, starts from the fact that treatment of serum by brief incubation with various colloids imparts to it a toxicity of the type we are considering. The process is accompanied by no cleavage of proteins perceptible by chemical means. Bordet has supposed that some protective or inhibiting substance is removed, and a latent toxicity unmasked; others, in vaguer terms, that the equilibrium of the colloidal solution is upset. Whether this change in the state of aggregation of the blood colloids is to be pictured as transmitting itself to those of the cell plasma is not clear. In any case it is not unreasonable to suppose that a change of this kind, which can excite a reaction in cells even when it takes place in body fluids brought into contact with them, would be even more effective if it occurred in the cells themselves. Now it is just a change of this kind, a change in the state of aggregation of the colloids, which is the one event which we are entitled to assume, with some confidence, as occurring in the protoplasm of the anaphylactic cell when specifically precipitating antibody there meets the corresponding antigen. So far as we know anything about the nature of the action, it is this. Langley, following Ehrlich, has accustomed us to visualize the relation of an active chemical substance, or drug, to the cell whose activity it modifies, by speaking of a "receptive substance" in the cell, for which the drug has a specific affinity. In the case of the ordinary drug action the existence of the receptive substance is a convenient hypothesis, summarizing certain observed facts. The action of the antigenic protein on the cell rendered anaphylactic to it, however, provides the one instance of a receptive substance which can be identified in the precipitating antibody. We have seen how this can be attached to and detached from the cell. It can be obtained in solution apart from the cell, and its mode of interaction with the drug, in this instance the sensitizing antigen, can be studied in the test-tube. When this receptive substance, *i. e.*, the precipitin is mixed with a high dilution of the drug, *i e.*, the antigenic protein, a change takes place; the colloidal complexes undergo a change in their state of dispersion, run together and ultimately form a flocculent precipitate. We must suppose that if the precipitin is in the protoplasm of a cell, and there meets the antigen to which it is specifically adjusted, a change in the state of dispersion of the protoplasmic colloids results. Now it is not impossible that such a change should initiate a fermentative change, leading to the formation of a substance having the histamine type of action or even of histamine itself. But we should still have to ask how histamine itself acts; why its production in the plain muscle cell, for example, should produce tonus. Of this, I venture to say, we know nothing. And in the existing state of our ignorance, it seems to me that the logical procedure is to interpret the unknown mode of action of these poisonous products from proteins in the light of what we do know concerning the mode of action of the specific antigen on the anaphylactic tissue; to suppose that histamine and the other substances having this

type of action also produce their action by initiating a change in the state of dispersion of the protoplasmic colloids; that it is a change of this kind, which, in terms of the different physiology of the different types of cell, causes contraction of the plain muscle, slackness and permeability of the capillary walls, and the whole group of phenomena which, with varying prominence in different species, constitute the complex which we know as the anaphylactic shock, and which histamine and the poisonous protein derivatives so faithfully reproduce.

This is the conception of the nature of the anaphylactic shock, and of its relation to the action of a group of natural proteinogenous poisons, which seems to me at present to be at once the most economical of hypothesis, and the most fruitful of suggestion for further investigation.

THE NATURAL HISTORY OF TYPHOID FEVER IN BALTIMORE, 1851-1919*

By WILLIAM TRAVIS HOWARD, JR.

THE WRITER'S STUDIES ON TYPHOID FEVER IN THE HEALTH DEPARTMENT

I entered the Health Department in the fall of 1915 and inherited the typhoid fever problem among others from Dr. Jones. The mortality rate per 100,000 for that year was 21.90. During 1916, I could not devote the necessary time to detailed study of histories of individual cases, but I was able to prepare the way for more complete histories than had before been obtainable and to take up more actively than had hitherto been practicable the study of the distribution of cases by five-day periods through a series of fifteen years, as well as to push the investigation of the relation of carriers to milk and to household and institutional epidemics. The latter work confirmed what was already known in the Health Department, that the bulk of the cases and deaths were reported in the summer and fall months, the peak coming usually late in August or in September, the fall in the autumn being gradual, and the rise and fall of the peak extending, as a rule, from July 15 to November 15. There was in the fifteen years rarely any evidence of a marked rise in spring, associated with freshets and so characteristic of the Great Lakes cities. With the greater facilities and leisure for this purpose than anyone had hitherto enjoyed, I set seriously to work in 1917 to determine, if possible, the most important sources of typhoid infection under the present conditions. The task was the easier because the number of reported cases to be investigated, as well as the number of deaths, had greatly diminished. The city officials, as well as the citizens, were greatly disappointed that typhoid fever had not almost completely disappeared after the new sewerage system and new water purification system were brought into use. It will be recalled that in the fall of 1915 the new water supply had become available and most of the connectible houses had been connected with the new sewerage system.

When my studies of typhoid fever in Baltimore began *in 1916,* it was estimated that about 60 per cent of the milk sold in Baltimore was pasteurized more or less efficiently. Some of these milk plants were well operated, both as to pasteurization of the milk and cleansing and sterilization of the milk utensils, including bottles and cans. In many of the pasteurizing dairies, including some of the largest, there were glaring faults. Most of the plants used the *holding system,* and on the whole their results were better than in those depending upon the *flash system.* In some of these latter plants, notably in one in which the proprietor did the pasteurizing himself, the results were very good. Many of the plants were not only hopelessly dirty and unscreened, but were structurally unfit for their purpose. The supervisor of pasteurizing dairies and his assistant, who overlooked these plants for the Health Department, were not only well trained but diligent and tactful. Their supervision consisted of general inspection, the inspection of automatically registered temperature charts, the insistence on proper cooling of milk, and the cleanliness of plants and utensils. They submitted a large number of samples of raw and pasteurized milk to bacteriological examination and spent much time in instruction. They won the cooperation of many of the dairymen, and beyond question, they did much to lift milk pasteurization from a purely commercial basis to a method of disease prevention. A large part of both the pasteurized and raw milk sold was delivered in churns, from which the milk was dipped with hand dippers for domestic use. Much of the milk sold in small grocery and milk stores was similarly served to customers. The condition of the churns and of the refrigerators in many of these stores beggars description. The regulation of the Health Department forbidding milkmen from removing bottles from houses harboring cases of communicable diseases until the bottles have been disinfected by the Health Department was being obeyed. The inspection of milk on its arrival in town to guard against skimming and watering was fairly well covered, as had been the case since the procedure was instituted in 1894. The far reaching system of dairy farm inspection with its cooperative relation with the deputy state medical officers, inaugurated by Drs. Jones and Blanck, was being carried out as well as could be expected.

The anti-typhoid work of the Health Department was extended in 1916 by a search for carriers in households, in restaurants and oyster-shucking establishments, and in connection with dairies and dairy farms. A canvas was made of restaurants, dairies, and dairy farms for workers who had typhoid fever. From the large number of such persons thus apprehended, routine cultures of the bowel and bladder dis-

* Continued from the August number of the Bulletin.

charges were made. Two carriers were found among dairy employees within the City. To one of them 10 cases of typhoid fever were traced. The other was a driver in a milk wagon who only handled the milk after it was bottled and to whom no cases of typhoid fever had been traced. Both were withdrawn from milk work. Workers on dairy farms suspected of being carriers, either came to the laboratory to furnish the desired specimens for culture, or the specimens were collected by the deputy state health officers. Two suspected carriers who had had typhoid the year before refused to be cultured. An epidemic of 24 cases of typhoid fever among the users of the milk from the dairy farm on which they lived subsided at once after the milk from this farm was excluded from the City. A total of 34 cases of typhoid fever was traced to milk during 1916. Four cases in one household and 1 case in another were traced to one carrier, a butler who had had typhoid fever fifteen years before. Nine cases in a college were traced to a female cook in a restaurant who proved to be a carrier. Another carrier, a cook in a private family, was apprehended. To her were attributed 2 cases of the disease, one occurring in 1914 and the other in 1915. In all, eight carriers were apprehended in 1916. Including the milk epidemic presumably due to one or two carriers on a dairy farm, nearly 30 cases of typhoid were traced to carriers this year.

All the remaining wells within the City were investigated by chemical and bacteriological methods, and quite a number showed evidences of pollution and were closed. Dr. Stokes isolated *B. typhosus* from the water of one such well that had been suspected as the source of infection of 4 cases of typhoid fever. A number of wells at roadside and fishing resorts near the City, frequented by Baltimoreans, were found to be polluted and reported to the State Board of Health.

One hundred and twenty-five cases of typhoid fever were classified as "infected out of town." Some of these were residents of the City who had contracted the disease while away for work or pleasure—many of them at summer resorts. Nine out of fifteen members of a baseball team developed typhoid fever after drinking water from a known polluted source at Grafton, West Virginia. Of the 110 Baltimoreans infected outside the City, 101 were infected in rural Maryland, and of the whole number of such outside infections, 106 occurred in the period of greatest incidence of the disease, as follows: July, 19, August, 23, September, 16, and October, 18.

Of the 776 reported cases, a satisfactory explanation of the source of infection was traced in 178, or nearly 22 per cent.

In addition to a search for carriers, attempts were made for better control of the milk supply, the elimination of polluted wells, and the prosecution of the fly nuisance through the newspapers, various civic bodies, certain insurance companies, the Street Cleaning Department, the health wardens, the nurses, and the Police Department. Instructions as to how to avoid typhoid fever, both at home and abroad, and urging anti-typhoid inoculations were not only distributed in the homes of cases of the disease, but through the medium of the newspapers, and circulars left in the houses. The latter was accomplished very largely through the collecting agents of industrial insurance companies. Physicians were requested to urge the general use of anti-typhoid vaccine, especially in households with recent cases of disease. The time was ripe this year, too, to prosecute the grading and smooth paving of the private alleys of the City, so long a nuisance in promoting the breeding and feeding of flies. This work, begun in 1916, was more than half completed with the paving of about one half of the 3000 such alleys, when the work was temporarily abandoned on account of the war.

In *1917*, the mode of infection was more or less convincingly traced in 225, or 41.17 per cent of the total number of 544 reported cases. The seasonal distribution of both cases and deaths remained relatively the same as in previous years. One hundred and forty-two, or 26.10 per cent of the reported cases, were classified as infected outside of the City (14 of these came from ships); 75, or 13.78 per cent of the total number of cases, were infected in rural Maryland; and 53, or 9.74 per cent, contracted the disease in other states. Table 4

TABLE 4

IMPORTED CASES AND OUT-OF-TOWN INFECTIONS, 1917

Maryland counties		Other states	
Baltimore	29	Massachusetts	1
Anne Arundel	15	Maine	2
Kent	9	West Virginia	2
Harford	4	Delaware	3
Worcester (Ocean City)	4	North Carolina	3
Caroline	3	New York	7
Frederick	3	New Jersey	9
Dorchester	2	Pennsylvania	13
Carroll	2	Virginia	13
Charles	1		53
Prince George's	1	Ships	14
Wicomico	1		67
Allegany (Cumberland)	1		75
	75		142

gives the distribution of these cases according to counties and according to other states. A considerable number of the 13 cases attributed to Pennsylvania occurred in non-residents of Baltimore, who came here for medical treatment or for work and who were either sick at the time of their arrival or became so within a day or two thereafter. Of the 13 cases attributed to Virginia, 4 were among Baltimoreans who became sick at Virginia Beach, and several other patients came to Baltimore from Norfolk or Portsmouth to hospitals for medical treatment. Of the 75 cases attributed to rural Maryland, a very large proportion (44) contracted the disease while sojourning in Baltimore and Anne Arundel Counties for work or on vacation. Nearly all of these cases occurred in the warm weather, when typhoid fever is most prevalent in the country districts. Of the total number of cases, 43, or 7.9 per cent, were traced to milk, there being three clear cut milk epidemics during the summer, all of which were traced to infection of the milk in dairies within the City. In 15 cases, a history of contact with recent cases within the household was obtained,

and in 6 further cases the infection was traced to hospitals in Baltimore, making a total of 21, or 3.85 per cent of cases traced to contact with clinical cases of typhoid fever. Fifteen cases occurred in families in which one or more members, occasionally unrelated inmates, had had typhoid fever one or more years before—the interval ranging from thirty years to one year. These previous cases of typhoid fever occurred in mothers, five times; grandmothers, twice; sisters, twice; brothers, four times; and fathers and wives, once each. In several of these families, there had been repeated cases. In two of the fifteen families, carriers were demonstrated by culture methods. One of these families is of particular interest, there having been, during a period of seventeen years, four outbreaks of typhoid fever in three generations with a total of 8 cases. The grandmother had contracted the disease seventeen years before; the next year, her two sons, her son-in-law and daughter and an unmarried daughter, all living in the same house with her, developed typhoid fever. In June, 1916, this woman's grandchild, aged 7 years, who lived in her house, developed the disease. On January 2, 1917, a second grandchild, a sister of the first, was reported as a case of typhoid fever. The whole family was cultured on January 8, 1917, and the grandmother, the unmarried aunt, and the child who had had typhoid fever in June of the previous year, were all three found to be carriers. Another carrier was found in the person of the mother of a boy who had had typhoid fever the previous year. In this year, eleven additional proven carriers were added to the Health Department's list, making a total of nineteen carriers under observation. In connection with contact infection, 4 cases gave a history of having had typhoid fever before, and it is suggested that these were examples of self-infection. One of these cases occurred within eight months, and a second within twelve months of the primary attack. One of the cases was treated in both attacks at the Johns Hopkins Hospital, the first attack occurring in August, 1908, and the second in January, 1917. In the fourth case, the attacks were six years apart.

In 1917, the morbidity rate per 100,000 was 83.79. Notwithstanding the fact that 24 deaths, or 26 per cent of the total of 92 deaths, occurred among typhoid fever patients brought to Baltimore hospitals for treatment from rural Maryland, the mortality rate fell to 14.17 per 100,000. There were 152 cases officially recorded by the State Board of Health against the counties from which they came, among whom the cases fatality rate was 15.85 per cent. The annual case fatality rate, excluding the above, but including cases and deaths of persons from other states than Maryland, was 11.75 per cent.

In *1918*, there were 302 cases and 73 deaths from typhoid fever, a decrease of 45 per cent in the cases and 28.7 per cent in the deaths as compared with 1917. The distribution of cases and deaths by months followed the same course as in previous years, with the exception that during October, the period of the influenza epidemic, the number of deaths from typhoid fever was disproportionately high, there being a total of 17 deaths for this month against 11 in September and 1 each in November and December. The number of out of town infections of Baltimore residents on trips, and of imported cases, was 53, or 17.55 per cent of the whole. In 39 cases, or 12.58 per cent, infection was attributed to contact with recent cases in the households. Five cases in one family were traced to a carrier and there was one case of laboratory infection. In addition to these, 16 cases were probably infected outside the City, and in 20 cases this mode of infection was a possibility. Twenty-two cases, or 7.9 per cent of the total number, resided in the same house with persons who had had typhoid fever previously, at times varying from thirty years to one year. Of the 24 possible infectors for this group of cases, there were 16 females and 8 males, but 2 of the possible male infectors were associated with possible female infectors in the same house; that is, in two of the households, the father and sister of the patients had had the fever. The 16 possible female infectors showed the following relationships to the patients: mother in 9 cases, sister in 3, wife in 1, unrelated female in 3 cases. The relation of the male infectors to the patients was: father alone, twice; father with sister, twice; brother, twice; husband, once; unrelated male, once. One father, upon whom the carrier state was proven by culture, was probably responsible for 5 cases among his children. In the latter part of July and the early part of August, the five children of a police officer came down in rapid succession with typhoid fever. The father, who had had typhoid fever fifteen years before, proved on culture to be a fecal carrier. No other source of infection for these children was ascertainable. A large proportion of these probable carriers were cultured, but only the one above mentioned was proven to be a carrier. Two additional carriers, to whom no cases were attributed, were apprehended. This year again, 4 cases presented a previous history of having had typhoid fever. The case of laboratory infection occurred in a medical student who swallowed typhoid bacilli sucked up through a pipette from a bouillon culture while working in a bacteriological laboratory.

The morbidity rate fell to 45.82 and the mortality rate to 11.08 per 100,000. Fourteen, or 19.18 per cent, of the 73 deaths from typhoid fever registered against Baltimore occurred among patients from rural Maryland sent to the Baltimore hospitals for treatment. There were 94 such cases against the 152 in the previous year. The case fatality rate among these cases was 14.9 per cent. During 1918, there were 59 deaths from typhoid fever properly chargeable to Baltimore, giving a case fatality rate of 17.2 per cent. As previously pointed out, the case fatality rate was influenced in great degree by the influenza epidemic in October, when there were 17 deaths among typhoid fever patients.

In *1919*, there was a large annexation of territory, in part closely built up, with a population for which no accurate figures are as yet obtainable. On the basis of the police census taken in the spring of 1919, it was estimated at 73,000, the proportion of negroes being much smaller than in the old City. The Bureau of the Census authorized the Health Department, for statistical purposes, to add about 60,000 for the annexed territory to the estimated population of the old

City. As the inclusion of this territory and its unknown population in the last year of the series would make accurate comparison of the course of the disease in former years impossible, for the purposes of the present study, the figures for the old City (old twenty-four wards) and for the Annex (new four wards) have been kept separate. The figures used in the graph are those derived from the old twenty-four wards. The problems of typhoid fever in the annexed territory will, therefore, be considered separately.

In the old twenty-four wards, the distribution of cases and deaths by months did not vary materially from the usual. Of the 293 reported cases, the modes of infection were traced as follows: 50, or 17.06 per cent, including definite imported cases in non-residents of Baltimore, and Maryland outside of Baltimore, were infected outside of the City; 36, or 12.78 per cent, occurred as secondary infections from recent previous cases in households and hospitals; 12, or 4.09 per cent, were infected by polluted wells and streams (2 cases were traced to wells at Curtis Bay and 10 to drinking the water of Gwynn's Falls); and 2 cases, or 0.67 per cent, were traced to laboratory infection. A total of 100 cases, or 34.05 per cent of the whole, were explained with reasonable certainty. A probable explanation was obtained for 52, or 17.73 per cent of the cases: 23, or 7.84 per cent, due to infection from remote cases in households; 5, or 1.70 per cent, due to neighborhood infection (from recent cases, probably through the medium of flies); and 23, or 8.19 per cent, due to infection outside the City. In addition, there were 14 cases, or 4.74 per cent, that were possibly infected outside the City. Altogether, an explanation, more or less satisfactory, of the mode or source of infection was obtained in 166 cases, or 56.52 per cent.

The cases attributed to infection from Gwynn's Falls occurred in ten children, members of a Sunday School in Southwest Baltimore (twenty-fourth ward) who took part in a picnic at Gwynn's Falls Park. Some twenty of the children went wading in Gwynn's Falls and, the day being very warm, drank the water of this stream. The cases of typhoid fever were restricted to those who so used the stream, and careful inquiry revealed no other source of infection. Half of those exposed to this source developed the disease. The stream at this point receives a considerable body of household sewerage, and there had been cases of typhoid fever in this area during the summer. The stream is also open to serious pollution above this point. It is of interest, that, as in 1918, no cases of typhoid were traced to milk. Two carriers were apprehended, making a total of twenty-four under restriction by the Health Department.

The morbidity rate was 43.79 per 100,000. There were 49 typhoid deaths in the old city, including 8 non-residents infected outside the City of whom 5 were from rural Maryland. The mortality rate was, therefore, 7.21 per 100,000. The mortality rate, less the 5 deaths among the 34 cases brought from rural Maryland to Baltimore hospitals for treatment, but including 3 other deaths of non-residents (a seaman, a person from Delaware, and another from West Virginia), was 6.58. The mortality rate among Baltimoreans alone was 6.28. The case fatality rate, based upon the 49 deaths occurring in the old twenty-four wards, and especially chargeable against Baltimore, was 16.72 per cent. The case fatality rate among the 34 cases from rural Maryland brought to Baltimore for medical treatment was 14.80 per cent. The case fatality rate for Baltimore minus these 5 deaths was 15.01 per cent. A more accurate picture of the case fatality rate, however, is obtained by dividing the whole number of cases reported in Baltimore in 1919 into the total number of deaths occurring among these cases not within the calendar months, but within a reasonable time afterwards. Of the 293 cases reported 41 died within the year 1919 and 3 between January 1 and May 1, 1920—a total of 44—giving a case fatality rate of 15.01 which happens to be exactly the same as that obtained when the deaths of patients from rural Maryland were left out of consideration. Approximately the same case fatality rate (14.80 per cent) is obtained by dividing the total deaths, including those of all non-residents dying in Baltimore, by the total number of cases reported in Baltimore, plus the cases brought into the City from rural Maryland for treatment (293+34=327). It would appear that a case fatality rate of about 15 per cent is correct for Baltimore (old twenty-four wards) in 1919.

Just as in the annexation of 1888, so in that of 1919 the City annexed a territory, much of which was characterized by insanitary conditions and with a high typhoid rate. In certain parts of this area, as in Roland Park, sanitary conditions were better than in the City itself. According to data kindly furnished me by Dr. C. Hampson Jones, when Chief of the Bureau of Communicable Diseases of the State Department of Health, there were 128 reported cases of typhoid fever in 1918 in the area annexed in 1919. A large proportion of these cases, 48, were in Highlandtown; 31 were in Brooklyn; and 22 in Curtis Bay. There were 10 cases in Arlington, 6 at Mt. Winans, and 5 at Hullsville. The water supply of Highlandtown is derived from the grossly polluted Herring Run, but is treated with chlorine. Upon examinations made in the laboratories of the Baltimore Health Department during 1919, this water supply showed evidence of marked pollution. In at least one milk epidemic in Baltimore in 1917, studied by me, cases of typhoid fever occurred in Highlandtown among customers of the infected dairy in the old City. There is every reason to suppose that milk has in the past played at least as important a part in spreading typhoid fever in Highlandtown as in the old City. The sewerage system is as crude as it formerly was in Baltimore. Much of Brooklyn and Curtis Bay proper have pure water supplies, filtered water from the Patapsco in the former and artesian well water in the latter. Both are, however, in large part without sanitary sewerage disposal; privies and cesspools being still largely in use. Overflowing privies and cesspools are common. In the summer of 1918, there was an extensive milk outbreak of typhoid fever in Brooklyn and Curtis Bay, traced to a single dairy. Mt. Winans and Hullsville are comparatively small places, in which the water is derived

from ordinary wells, and the common privy is still in use. Many of the smaller villages and settlements in the northwestern portion of the annex have pure water, supplied either from artesian wells or from the Avalona supply of the Baltimore County Water Company. Good water is served to Mt. Washington, Roland Park, a part of Arlington and Govans, and to a part of the developments on the Harford Road. In much of Arlington, Govans, and the settlements along the Harford Road, there are no proper sewers, and cesspools and privies are situated so as to pollute the wells from which drinking water is obtained. So, in much of the newly annexed territory, the City is confronted with insanitary conditions fraught with danger.

With the annexation, the City's laws and regulations in regard to milk became immediately applicable to the new wards, and fortunately in 1919 there were no milk epidemics. There was a total of 65 cases of typhoid fever reported in the four new wards in 1919, in comparison with 128 the previous year. Of the 65 cases in the Annex, 8, or 12.31 per cent were classified as infected outside of the City; 6, or 9.23 per cent, were attributed to contact with recent cases in households; the same number, to contact with remote cases; 12, or 18.46 per cent, to infection from wells or springs; and 2, or 3.07 per cent, to neighborhood infection, probably by flies. No cases were traced to milk. It will be noted that in the Annex a somewhat larger proportion of the cases were explained than in the old twenty-four wards.

On an estimated population of 65,000, there was a morbidity rate of 100 per 100,000. There were 9 deaths. The mortality rate was 13.84 per 100,000, and the case fatality rate was 13.84 per cent. The mortality rate of the new four wards was nearly twice that of the old twenty-four wards.

SUMMARY OF INFORMATION IN REGARD TO SOURCES OF INFECTION OF TYPHOID FEVER, 1916-1919

Sources of Infection in Reported Cases.—In Table 5, the results of the investigations of 1916 to 1919, inclusive, on the sources of infection of reported cases are set forth. It will be noted that the proportion of cases for which no explanation of the mode of infection is determined falls steadily from 1916 to 1919. This is to some degree due to the fact that in the first two years the data in regard to probable and possible outside infections, having been destroyed by fire, are lacking in the table.

Attention is directed to the very large proportion of cases certainly infected outside the City, varying from 16 to 26 per cent in the four years. These include the imported cases occurring among non-residents from other states or from foreign countries. The great bulk of them, however, are represented by residents of Baltimore who contracted the disease outside of the City while away for work or vacation. It will be seen that the percentage of cases infected outside of the City is fairly constant for this period. In addition to this group, there are two other comparatively large groups in 1918 and 1919, one in which infection outside of the City was probable but not certain, and a second in which it was possible, due to the fact that the individuals had been out of town for one or two days only within three weeks or a month before the beginning of the illness.

The very much larger percentage of cases traced to contact with recent cases in households and hospitals in 1918 and 1919 over that of 1917 is undoubtedly to some extent due to more adequate histories obtained in the last two years. Cases were placed in this group only after the most critical consideration; no cases were so assigned unless there was a definite interval of a month or more between the beginning of the illness of the first and succeeding cases, or cases, and only when any other mode of infection could be excluded with reasonable certainty. A surprisingly large percentage of these

TABLE 5

THE SOURCES OF INFECTION OF TYPHOID FEVER FOR 1916 TO 1919, INCLUSIVE

Sources of infection	1916		1917		1918		1919			
	Cases	Percentage of cases	Cases	Percentage of cases	Cases	Percentage of cases	24 wards cases	Percentage of cases	Annex cases	Percentage of cases
Infected out of city, including imported cases.	125	16.14	142	26.10	53	17.55	50	17.06	8	12.31
Contact with recent cases in households and hospitals.	..	....	21	3.86	39	12.58	26	12.28	6	9.23
Contact with remote cases in households.	..	....	15	2.75	22	7.90	23	7.84	6	9.23
Neighborhood infection.	..	....	..	....	..	....	5	1.70	2	3.07
Springs, wells, and streams.	4	0.51	..	....	..	....	12	4.09	12	18.46
Carriers.	14	1.80	..	....	5	1.65	..	....	..	....
Milk	34	4.52	43	7.90	..	....	..	...	..	....
Laboratory	..	....	..	....	1	0.33	2	0.67	..	....
Self-infection	..	....	4	0.73	4	1.32	..	....	..	....
Probable outside	..	....	..	....	16	5.22	24	8.19	8	12.31
Possible outside	..	....	..	....	20	6.26	14	4.74	1	1.53
Unexplained	597	77.13	319	58.83	142	47.02	127	43.34	22	33.84
Total cases	774	100.00	544	100.00	302	100.00	293	100.00	65	100.00

cases occurred among individuals admitted to the general wards, or semi-private wards, of hospitals to be treated for other affections and who developed the disease three weeks or more after admission. In nearly all of these cases, definite, associative contact with typhoid fever cases in the wards could be traced, and in every instance there were cases of typhoid fever in these hospitals at the time of the patient's stay. These cases occurred in four large hospitals.

It will be noted that infection from springs and wells played an unimportant part, there being 4 cases traced to one polluted spring in 1916 and 2 such cases in 1919. In the latter year, there was an epidemic of 10 cases among children who drank the polluted water of Gwynn's Falls.

It is significant that in 1916 and 1917 a large number of cases in definite epidemics were readily traceable to infec-

tion through milk, whereas in the last two years, after the new milk ordinance was put into force, no cases were traced to milk.

The 3 cases traced to infection in bacteriological laboratories in 1918 and 1919 were, of course, fortuitous and have no important bearing upon the question under discussion.

Under self-infection are included the 8 cases noted in the four years, in which the individuals gave a history of having had typhoid fever before and which are included in the table as possibly explained cases. Concerning the possible importance of this source of infection, much can, of course, be said for and against.

There are included in the group under carriers, all cases in which infection through means other than milk is attributed to proven carriers. As will be seen later, although diligent search was made, comparatively few cases in households were traced to proven carriers during the last three years. Allusion has been made to the extensive investigation conducted since 1916 for the apprehension of possible carriers among food handlers. Including these and possible carriers in households, in the four years, 426 individuals were submitted to the test made by culture of both feces and urine. The methods in use for this purpose are those of the U. S. Army Manual; *i. e.*, cultures from fluid stools or from urine on Endo's medium. The work was done by Dr. Stokes or by Dr. Hachtel, or under their immediate supervision. Twenty-four, or 5.26 per cent of these individuals, were thus proven to be carriers. There are now, therefore, twenty-four carriers under control of the Health Department.

Attention was drawn to the importance of infection from remote cases in households when searching for carriers in households, in which, according to the histories of reported cases, a member of the household, almost invariably a relative, was said to have had typhoid fever at some remote date. While from time to time the carrier state was established in many of these suspects by culture, more often even repeated cultures from both bowel and bladder discharges were negative. As my experience with proven carriers, especially bowel carriers, grew, I was struck with the fact that, as tested by cultures made in the ordinary way, there was great intermittency of the carrier state. In the case of a urinary carrier, a butler, there was very considerable intermittence of the emission of the typhoid bacilli. This man served in one family for eight years before any of them developed clinical typhoid fever. Suddenly four were attacked in short order, and his own grandson, who had been living with him for some years, developed the disease at the same time. Further accumulated evidence showed that in a very large proportion of the cases occurring in individuals living in households with proven carriers this association had been going on for years. It seemed to me likely that the infection, or non-infection, of the exposed must be determined by something more than chance contamination of food or drink, under conditions favorable for the multiplication of the typhoid bacilli in such articles to a degree necessary to make an infective dose for the victim, even if it be assumed that the susceptibility of the latter remained constant. Experience with typhoid carriers shows that in many instances at least a very favorable combination of circumstances, ruled to a very considerable degree by chance, must occur in order that they may successfully inoculate others with the typhoid fever disease. From my experience, it would appear that one of the factors involved in this combination is the intermittence of emission of the infective agent by the carrier. Another forcible factor must be the opportunity offered for infection by the character of the associative contact between carrier and victim. It is well known that carriers who handle food for others have better opportunities than have non-food handlers for transmitting typhoid organisms, at least in numbers adequate for producing clinical typhoid fever. Therefore, suspected carriers, the possible infectors of individuals living in households with them, should be shown to be peculiarly favorably situated in the character of their associative contact to transmit the infective agent, before any definite importance can be attached to them as the sources of infection. Unless this is done, and other possible sources of infection can be excluded with a reasonable degree of certainty, the association between cause and effect is based upon little more than suspicion. If it be shown that the emission of typhoid bacilli (as proved by the culture test) is intermittent among chronic typhoid carriers, and that the suspected carriers, supposed to be responsible for infecting household associates, occupy a relation in the household that favors transmission of the infective agent by food and drink and are in any large proportion of cases food handlers, the character of the evidence is raised to a much higher level.

Analysis of the observed cases during the three years, 1917 to 1919, inclusive, shows that of 68 such cases, including carriers and suspected carriers in households, 66.17 per cent were females, and the percentage of females to males was approximately the same in each year. (See Table 6.)

TABLE 6

SEX DISTRIBUTION OF PROVEN AND SUSPECTED TYPHOID CARRIERS IN HOUSEHOLDS

Year	Number of persons	Females	Males	Percentage of females
1917	15	10	5	66.66
1918	26	18	8	69.23
1919	27	17	10	62.96
	68	45	23	66.17

The forty-five female carriers or suspected carriers bore the following relationships to the typhoid fever patients: Mothers, 24; sisters, 12; wives, 3; grandmothers, 2; aunts, 1; domestic servants, 3. The twenty-three males in question held relationship to the patients as follows: Fathers, 9; brothers, 9; husbands, 3; uncle, 1; unrelated male, 1.

Of the whole number in this group of sixty-eight suspected carriers, in only six cases was the carrier state established by culture.

The argument that these suspected carriers were, at the time of the infection of the individuals associated with them in their households, true carriers and responsible for spreading the disease is supported mainly by the facts; first, that in none of the cases of typhoid fever was any other source of infection traceable; second, that as determined by ordinary culture methods, known typhoid carriers often show marked intermittence of emission of typhoid bacilli in their discharges; and third, that there was a great preponderance among the suspected carriers of mothers and sisters and other females most likely to be food handlers.

If the inference that typhoid bacilli carriers, both fecal and urinary, are often intermittent in the discharge of typhoid bacilli be correct, it naturally follows that it is erroneous and dangerous in public health administration to conclude, on the basis of a single or even several negative cultures, that an individual who has had typhoid fever is not a carrier. It would rather seem that, in order that a typhoid carrier may infect others, among the other various elements of chance that must fall favorably is the one that the carrier be in a period of emission of the infecting agent. It is now well established that, in a typhoid bacillus carrier, cultures may show this organism in large numbers in material removed by a duodenal tube and yet none appear in fecal cultures taken at the same time. If this be the case with fecal carriers, even much more weight is to be placed upon the evidence at hand in support of the theory of intermittency of the discharge of typhoid bacilli in the case of urinary carriers, for it is a comparatively simple matter to cultivate the typhoid bacillus, when present, from urine collected in a sterile container, for the chances of overgrowth of the typhoid bacillus by other organisms is slight, probably much less than is the case with material collected from the duodenum in bile-system carriers.

TABLE 7

SOURCES OF INFECTION OF TYPHOID CASES FOR 1919

	Old 24 wards		Annex		City as a whole	
	No. of cases	Per-centage of cases	No. of cases	Per-centage of cases	No. of cases	Per-centage of cases
Certainly explained						
Imported	14	4.74	..	....	14	3.91
Outside certain	36	12.28	8	12.31	44	12.29
Recent contact, homes and hospitals.	36	12.28	6	9.22	42	11.73
Laboratory	2	0.67	..	....	2	0.53
Gwynns Falls	10	3.41	..	....	10	2 79
Springs and wells	2	0.67	12	18.46	14	3.91
Total	100	34.05	26	39.99	126	35.16
Probably explained						
Remote cases	23	7.84	6	9.23	29	8.10
Neighborhood infection	5	1.70	2	3.07	7	1.95
Outside probable	24	8.19	8	12.31	32	8.93
Total	52	17.73	16	24 61	68	18.98
Possibly explained						
Outside possible	14	4.74	1	1.53	15	4.18
Total	14	4.74	1	1.53	15	4.18
Total explained	166	56.52	43	66.13	209	58.32
Total unexplained	127	43.34	22	33.84	149	41.62
Final total	293	100.00	65	100.00	358	100.00

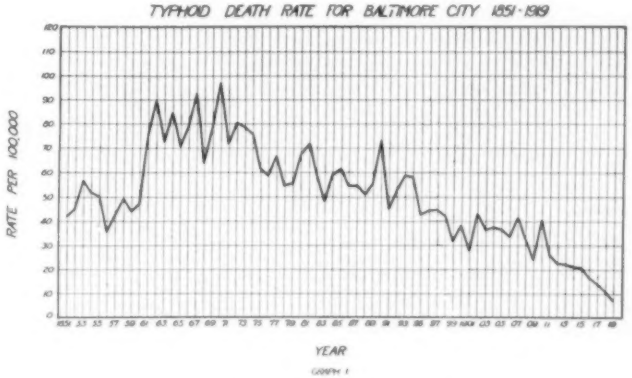
In Table 7, the results of the study of the sources of infection of the cases of typhoid fever, reported from the old twenty-four wards and the Annex during 1919, are set forth in greater detail.

STATISTICAL ANALYSIS

In this analysis, it is for the most part necessary to proceed under the assumption that the official figures of the Health Department for cases and deaths of typhoid fever are correct. It is realized that this assumption includes many sources of error. Unfortunately, the available data do not afford material upon which even approximate corrections may be based. The chief sources of error affect the period previous to 1890.

I. MORTALITY RATES

Total Population.—In Graph 1 are represented the annual mortality rates per 100,000 living population in Baltimore from 1851 to 1919, inclusive. For the sake of more accurate comparison, the rate for 1919 has been calculated for the old twenty-four wards of the City. It will be noted in the graph that for the first ten years the mortality rate averaged about 45 per annum. In 1861, there was a decided increase in the rate which continued at about 80 until 1871, since which time there has been an irregular but decided fall.



These rates have been calculated from the reported deaths from typhoid fever alone, with the exception of the years from 1879 to 1898, inclusive, in which the deaths ascribed to "typho-malaria" have been included. If the rates calculated from the deaths from gastric fever be added to the rates given for typhoid fever on the graph, from 1851 to 1875 inclusive, (the years of our period during which gastric fever, which was very likely typhoid fever, played a prominent part as a cause of death), the course of the curve for typhoid fever could not be materially changed; it would simply mean adding rather uniformly from 5 to 10 to the mortality rate from 1851 to 1875. Any important influence of typhus fever on the curve

for typhoid fever is rather remote for two reasons: In the first place, though typhus fever occurred endemically in Baltimore until 1857, its course was marked by distinct epidemic waves, and the wave which began in the early 40's subsided in the early 50's. In the second place, the fact that typhoid fever was first recognized by the Health Department in 1851 suggests very strongly that by this time the diagnosis between this disease and typhus fever was made with a fair degree of facility by local physicians.

The deaths from malarial fevers under various names, including bilious fever, present problems of greater difficulty in this connection. From the early medical literature of Baltimore, it is clear that bilious fever, or bilious remittent fever as it was usually called, was regarded by the medical profession as a form of malaria due to marsh effluvia and as synonymous with remittent malarial fever. It was commonly confused with yellow fever, which was regarded by many of the ablest physicians not as a separate disease but as a severe type of bilious remittent fever. To it was ascribed a very heavy mortality from 1812, when my records begin, until 1842. In 1851, bilious fever declined to 22.53 per 100,000, but during the next three years there was a distinct rise, succeeded by a considerable fall in 1856. By 1861, the rate per 100,000 had fallen to 15. After 1867, there was another rise, culminating in 1874.

If the rates for all the malarial diseases taken together were added to those for typhoid fever, the curve for the latter would show two distinct upward waves; one a very high short one, corresponding to the one evident on the graph between 1851 and 1856; the other a long wave, culminating in a very high peak in 1873. After 1874, the shape of the curve would not vary materially from that of typhoid fever as shown on the graph.

To remittent fever were ascribed comparatively few deaths before 1870, and the highest rate attained by this disease from 1851 to 1870 was 4.88 per 100,000. Intermittent fever was recognized as a separate disease from the beginning of my records for Baltimore. This disease ran a very irregular course, never reaching a very high mortality rate except in 1821 when the rate was 29.85 per 100,000, and, on the whole, its curve rises and falls with that of bilous fever. If all the deaths ascribed annually to intermittent fever were regarded as due to typhoid fever, the rate of the latter, from 1851 to 1866, would not be greatly altered, and its curve would be scarcely affected. Of all the varieties of malarial fever, this one is least likely to be mistaken for typhoid fever. From 1867 to 1874, inclusive, the rate for intermittent fever, and correspondingly the rate for all the fevers ascribed to malaria showed a decided rise.

In the above comparison, it is sought to bring out the facts that, if all the deaths ascribed to the different types of malaria, occurring between 1851 and 1874, inclusive, were classified under typhoid fever, the course of the curve for the latter would be changed in two respects; namely, the upward wave between 1851 and 1856 would be much higher, and the rise in the rates between 1861 and 1870 would have begun several years earlier and continued three years longer—denoting two epidemic waves of typhoid fever instead of one, the peak of the first in 1853 and that of the second in 1871. In reality, however, as the malarial fever deaths between 1866 and 1874 were ascribed chiefly to those types of the disease least likely to be confused with typhoid fever (intermittent and congestive fevers), the confusion of malaria with typhoid fever as a cause of death could not very materially have affected the curve as plotted from the deaths officially ascribed to typhoid fever. Nevertheless, two points are evident: First, that typhoid fever before 1851 was hidden, mainly at least, in this particular group of diseases; and second, that, after its emergence as a distinct disease from the mass of other diseases characterized by continued fever, it was for a long time confused with them to such a degree that the mortality rate for typhoid fever obtained from the official figures is probably much lower than the true rate until about 1890.

It is very possible that typhoid fever was not infrequently confused with dysentery and diarrhœa and with "inflammation of the bowels." As late as the first half of the 19th century, dysentery was thought by many physicians to be a form of malaria, with which it, in common with many other diseases, was so often complicated. Few of the population, in certain sections of the City at least, escaped malarial fever at this time. It is not unlikely, therefore, that cases of atypical dysentery, especially when complicated with malaria, were mistaken for typhoid fever. "Inflammation of the bowels," from 1840 to 1897, and diarrhœa, from 1875 to 1900, were credited with a considerable number of deaths, and it is probable that these two more or less indefinite affections were confused with typhoid fever. Just to what degree the confusion with typhoid fever of deaths classified under these three categories affeceted the typhoid rate, it is impossible to determine.

On the other hand, it would be grossly erroneous to assume that all the deaths officially ascribed to typhoid were actually due to this disease. There are ample reasons for concluding that many deaths from pneumonia, pleurisy, empyema, influenza, appendicitis, suppurative processes of the liver and the biliary and urinary tracts, acute miliary tuberculosis, acute phthisis, and the various cryptogenic suppurative infections, as well as pyæmia and septicæmia, following wounds and injuries, including those caused by surgical procedures—the large group of affections often characterized by the "typhoid state"—were attributed to typhoid fever. Before the isolation of para-typhoid fever as a distinct disease, deaths from this affection were probably classed under typhoid fever, as they are even now under the international classification of causes of death.

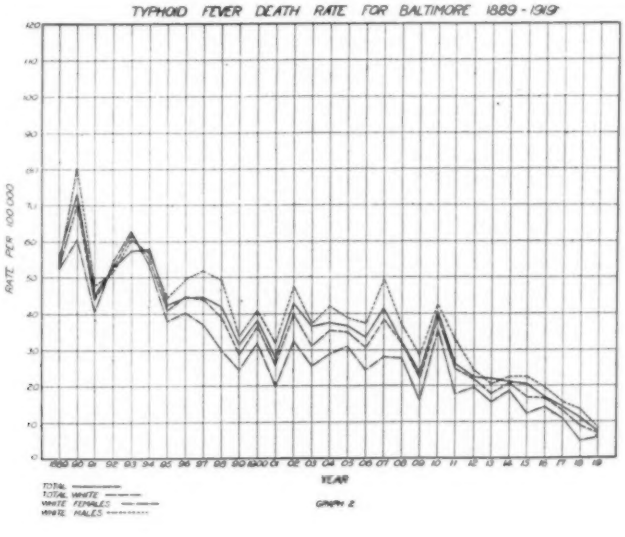
Another source of error to be taken into consideration is the influence upon the death rate of typhoid fever of deaths of non-residents, particularly those occurring among cases brought to hospitals in Baltimore for treatment. No separarate account of these was kept until 1917. In the three year period, 1917 to 1919, these deaths accounted for 20 per cent of the total deaths from typhoid fever in the City. The deaths

within the City of non-residents from other States more than counterbalance the few deaths from typhoid fever in Baltimoreans away from home. It is probably warrantable to assume that this source of error was of relatively small importance until transportation was made easier by improved roads and the use of the automobile from about 1905. Since this period, probably from 15 to 20 per cent of the deaths recorded from typhoid fever in Baltimore occurred among cases imported from rural Maryland for treatment. As early as 1890, the Baltimore hospitals received many cases of typhoid fever from Sparrows Point, and it must be recalled that certain thickly settled parts of the adjacent suburbs, Highlandtown for instance, were readily accessible to some of the hospitals as many parts of the City.

From all the evidence at hand, therefore, it seems probable that the *shape* of the true mortality curve for typhoid fever for Baltimore, between 1851 and 1919, if we had the data for calculating it, would at least closely approximate that derived from the official figures, although the rates would be much higher in the earlier years.

White Population.—Graph 2 shows that the total rate for the white population exceeds the rate for the whole population only in 1893 and 1896. It closely approximates it in 1897, 1908, 1910, and 1916.

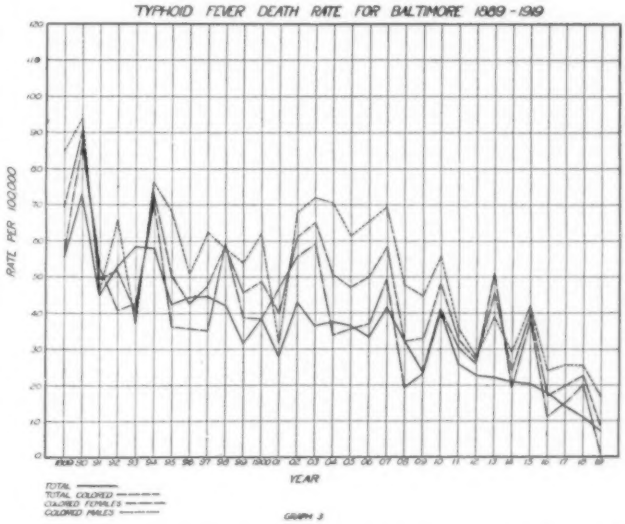
The rate for white females is well under the rate for the males, as well as under that for the total population, but it



exceeds the rate for the total population in 1893. In general, the rate for the females follows accurately the rise and fall in the other rates, particularly the rate for total white and total population. In some few years, it falls or rises more abruptly than the other rates.

The rate for white males alone is uniformly higher than the other rates, with the following exceptions; in 1892, when it is slightly lower than the rest, and in 1894 and 1913, when it is slightly lower than the rate for the total population. This rate rises and falls uniformly with the other rates and the curves approximately fit each other.

Colored Population.—The curves for the colored population in Graph 3 are much more complicated than those for the white. That for total colored falls considerably below

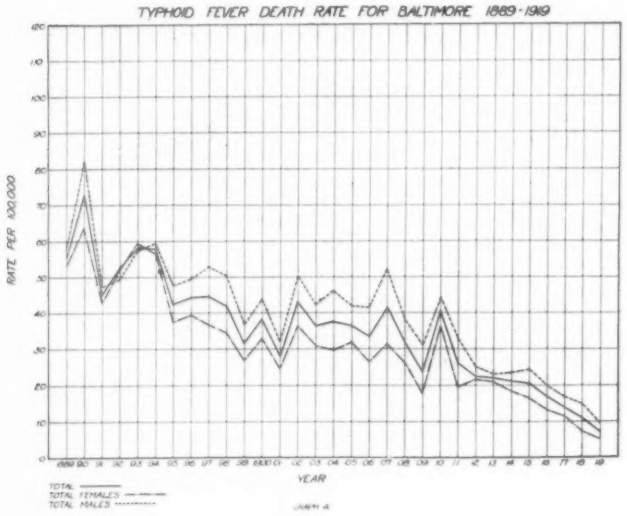


the total rate in 1893, the year in which the rate for the total white population was above the total rate. The course of the curve for the total colored population in relation to that for the total rate is in the main quite different, in that it often fails to follow the dips and elevations in the total rate, but even when it does follow these, it is apt not to conform closely. In 1913, the year in which the total white rate dips considerably below the rate for the total population, the total colored rate, on the contrary, shows a marked peak. In 1914, when there is but a very small fall in the total rate, there is a considerable drop in the total colored rate. In 1915, this is compensated for by a considerable peak in the colored rate. In 1916, the colored rate falls slightly below the rate for the total population. In 1917 and 1918, there is again a decided rise in the total colored rate, with a marked drop in 1919. The highest rate for the whole series of years is the colored rate for 1890 which reaches 90.13 per 100,000 while the highest rate for total whites is 69.93 per 100,000 in the same year. As would be expected from the different proportions which they hold in the population, the curve for the negro rate is nothing like so uniform as that for the white rate.

The rate for colored males is uniformly higher than that for colored females, except in 1893, 1901, and 1913, when the latter is markedly higher. The peaks and depressions of the two curves for colored males and females generally follow each other. There are, however, some notable exceptions.

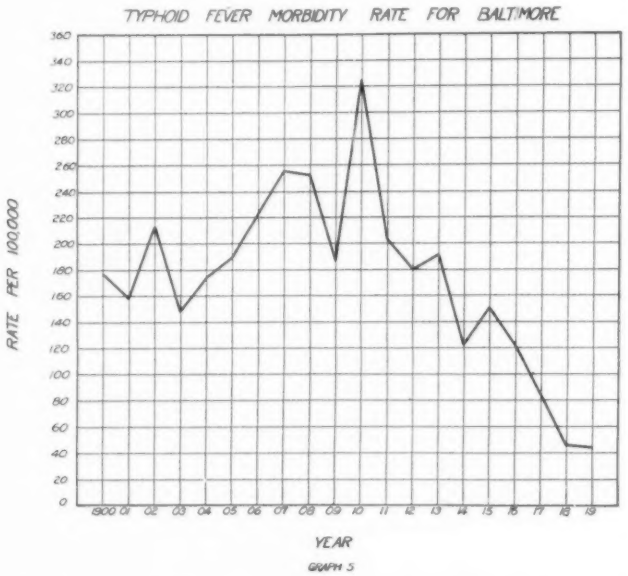
The curve for female colored is very irregular, but in general, it is higher than that for the total population. It is lower than the latter in 1895, 1896, 1897, 1904, 1908, 1909, 1914, 1916, and 1919. In some of these years, it is very noticeably lower.

Males and Females.—There is remarkable uniformity in the three curves plotted on Graph 4 which fit much more closely throughout than the curves previously considered.



The rate for females is slightly higher than that for both the total population and for total males in 1893, but with this exception, it is uniformly lower, and generally considerably lower than the rate for males. The rates for the two sexes vary as much as 20 per 100,000.

These graphs show very clearly throughout the period under consideration, from 1889 to 1919, inclusive, that the total rate



has been markedly influenced by both sex and color, that the presence of negroes tends to raise the general rate, and that the presence of females tends to lower it. In other words, since the rates for the colored population are much higher than those for the white, and the rates for females of both races are lower than those for males, the total rate is influenced favorably by a low proportion of negroes and a high proportion of females in the population.

II. MORBIDITY

Although typhoid fever was by law made reportable in 1894, it was not until 1900 that the cases were reported with reasonable accuracy. In Graph 5 are given the morbidity rates per 100,000 inhabitants from 1900 to 1919, inclusive. It will be noted that the morbidity curve corresponds in a general way with the mortality curve throughout its course, and that from 1910 to 1919 the two curves fit with a fair degree of accuracy. Unfortunately, data for comparing the morbidity in the colored and the white races are at hand for only 1918 and 1919. Comparison for these two years by sex and color is shown in Table 8. It will be noted that the morbidity rate

TABLE 8

TYPHOID MORBIDITY RATES PER 100,000 BY RACE AND SEX FOR 1918 AND 1919

Year	Baltimore							Rural Maryland		
	White males	White females	Total white	Colored males	Colored females	Total colored	Total morbidity	Total white	Total colored	Total morbidity
1918.....	61.32	33.71	47.05	29.44	47.51	39.11	45.82	.....	.....	.....
1919.....	50.89	41.09	45.83	41.34	25.23	35.62	43.79	.....	.....	.....
Average for 1918 and 1919.	56.10	37.40	46.44	35 39	36.37	37.37	44.81	152.24	262.88	172.85

for the colored is decidedly lower than that for the white population, just the opposite to that which obtains for rural Maryland, as is shown by the figures calculated from data kindly furnished me by Dr. John S. Fulton, Secretary of the State Board of Health. The difference in the ratio of incidence of the disease in the two races in Baltimore as compared with rural Maryland is, to some extent, explained by the larger proportion of imported cases and of out of town infections among the white population in Baltimore. So far as may be judged by the figures for these two years, the incidence of the disease is not only greater in the white race than in the colored, but is greatest in white males, and greater in white females than in either colored males or females.

III. CASE FATALITY RATE

Much interest attaches to the case fatality rate of typhoid fever in Baltimore, among other reasons because it has been used as a measure in estimating the degree of completeness of case reporting. Any conclusion in regard to case fatality must depend upon the accuracy with which cases and deaths are reported.

In his early studies on typhoid fever, Dr. C. Hampson Jones expressed the opinion, based upon the assumption that 10 per

cent is a normal average case fatality in this disease, that the number of reported cases fell far short of the actual number existing—in some years by nearly 70 per cent. That the reporting has not been perfect is beyond question.

There are several fallacies not uncommon in studies of this character which should be pointed out. In the first place, it is difficult to collect the information on which to set a correct standard for the case fatality rate for any disease, and this is especially difficult for typhoid fever. One must have a large number of cases in which the diagnosis is established with accuracy by the use of refined laboratory methods, and the chance of burying under typhoid fever individuals dead of other diseases must be eliminated by submitting to autopsy all the fatal cases in the series. An important source of possible error in Baltimore is due to the fact that many of the deaths registered as due to typhoid fever occur, as has already been pointed out, among cases brought from the counties of Maryland for treatment in the city hospitals, the reported cases being credited to the place of origin by the State Board of Health and the deaths then being recorded against Baltimore; *i. e.*, cases are transferred, but the deaths are not. The error lies in the fact that the divisor is disproportionately small. It is obvious, therefore, that for the purpose of establishing even an approximately correct case fatality rate, these two groups of cases and deaths must be reckoned, either combined or separately; *i. e.*, the county cases must be added to the divisor or the deaths among them must be subtracted from the dividend. The importance of this is well illustrated in Table 9, from which it will be seen that the average case

TABLE 9

CASE FATALITY RATES

Year	I			II			III			IV		
				Baltimore and rural Maryland total cases and deaths			Rural Maryland			Baltimore		
	Baltimore cases	Baltimore and rural Maryland deaths	Percentage case fatality	Cases	Deaths	Percentage case fatality	Cases	Deaths	Percentage case fatality	Cases	Deaths	Percentage case fatality
1917.....	544	92	16.91	696	92	13.22	152	24	15.79	544	68	12.50
1918.....	302	73	24.17	396	73	18.43	94	14	14.89	302	59	19.54
1919.....	293	49	16.72	327	49	14.98	34	5	14.71	293	44	15.02
Total and average.	1139	214	18.79	1419	214	15.08	280	43	15.36	1139	171	15.01

I. Official figures and case fatality rates.
II. Official figures and case fatality rates as corrected by addition of cases from rural Maryland.
III. Figures and rates for rural Maryland cases treated in Baltimore, and deaths among such cases.
IV. Correct figures and case fatality rates for cases and deaths properly chargeable against Baltimore.

fatality rate for the three years, 1917 to 1919, inclusive, calculated without making this allowance, is 18.79, per cent, while a much lower rate is obtained either by adding the total cases reported from rural Maryland to the divisor, or by subtracting the deaths among them from the dividend. It will be noted that the average for the three years for the cases and deaths occurring among Baltimoreans in Baltimore is 15.01 per cent. From Sections I, II, and IV of this Table, it is clear that the official annual and average annual case fatality rates for 1917, 1918, and 1919 (for the old twenty-four wards, as shown in Section I) are far from the correct rates. The source of error, of course, in Section I lies in the smallness of the divisor, from which is officially excluded the number of cases from rural Maryland, in proportion to the dividend in which the deaths occurring among these cases are included. In Section II, this error is removed by adding the cases in question to the divisor. Section IV gives the corrected rates for Baltimore based on actual cases and deaths reported, but excluding from the dividend the deaths occurring among the cases charged officially against rural Maryland. In Section III are given the correct rates for the cases from rural Maryland treated in Baltimore during the years in question. It will be noted that the average annual case fatality rates for Sections III and IV (*i. e.*, rural Maryland and Baltimore) are approximately the same.

It is evident that the annual case fatality rates derived from the deaths and reported cases officially charged against Baltimore are not the true rates. The method of deriving annual case fatality rates by dividing the number of cases into the number of deaths reported in a given calendar year is open to an error which may be considerable; not all the deaths that may occur among the cases reported in a given calendar year take place within that period of time. The early months of the calendar year will usually have deaths occurring among cases reported during the late months of the previous year,

TABLE 10

CASE FATALITY RATES, ACCORDING TO SEX AND COLOR, 1919

Baltimore, 1919, twenty-four wards

	Mortality	Morbidity	Percentage case fatality
Total white.....	35	259	13.89
Total colored.....	9	34	26.47
Total males.....	27	159	16.98
Total females.....	17	134	12.68
Male white.....	20	139	14.38
Female white.....	15	120	12.50
Male colored.....	7	20	35.00
Female colored.....	2	14	14.28
Total white and colored.....	44	293	15.01

and this process may be expected to continue throughout the series of years under consideration, and the last year in the series will almost surely lack some deaths. This error may be reduced to some degree by averaging the rates for a series of years.

A more accurate method is to take into account only the deaths occuring within the group of cases allowing a reasonable length of time for all the fatalities likely to happen within the group. This was done for the cases reported within the

old twenty-four wards during 1919. In the 293 such cases there occurred 44 deaths—41 during 1919 and 3 between January 1 and May 1, 1920. The case fatality rate thus derived, 15.01 per cent, happens to be the same for this single year as that for the average for the three years, as calculated by the usual method.

The accurate data for determining the influence of sex and color upon the typhoid case fatality rate in Baltimore are rather meagre, being limited to the year 1919. These are set forth in Table 10. On account of the smallness of the figures for some of the categories and the absence of correction for age, some of the percentages are perhaps subject to considerable probable error. Taking them at their face value, however, the risk rate of dying of clinically recognized typhoid fever is twice as great for colored as for white, somewhat greater for white males than for white females, and twice as great for colored males as for colored females, and nearly a fourth greater for total males than for total females.

SUMMARY OF THE KNOWLEDGE OF THE INFLUENCE OF DIFFERENT FACTORS ON TYPHOID FEVER IN BALTIMORE

From the mass of facts set forth in the preceding pages, it is evident that most of the various factors concerned in the spread of typhoid fever have acted continuously, but with varying degrees of force, during the whole period. The most important of these factors are:

(1) Water Supply.
(2) Crude Methods of Sewerage Disposal.
(3) Milk.
(4) Importation of cases and carriers in:
  a. Immigrants.
  b. Military.
  c. Isolated Individuals.
(5) Infection contracted without the City.
(6) Associative Contact with Recent Cases and with Remote Cases; *i. e.*, Carriers.
(7) Flies.
(8) Bathing in Polluted Waters.

Water Supply.—The water supply was seriously polluted from the earliest times until 1911, but the degree of pollu- has varied at different periods and with the sources from which the water was obtained. In 1850, at least a half and probably two-thirds of the drinking water was obtained from wells within the City. The proportion obtained from these sources was notably reduced after the City took over the water supply from the private company and provided a more abundant and better distributed supply after the establishment of the Lake Roland Dam on Jones' Falls in 1862. Nevertheless, a large part of the inhabitants clung to the internal water supply from springs and wells until long after this date. Through the efforts of the Health Department, these were practically eliminated in the old City of that period by the time of the annexation in 1888. It is probable that the Lake Roland watershed became heavily infected with typhoid fever during the Civil War. The completion of the Gunpowder water supply system, with its numerous storage reservoirs, and the substitution to a very great extent of water from this source for the old Lake Roland water supply must have favorably influenced the course of typhoid fever for a considerable period after 1881. Indeed, with the exception of a few years after the annexation of 1888, the whole trend of the typhoid fever curve was distinctly downward for the next twenty years. After 1901, the water supply again became more and more polluted, and there was an upward wave in the typhoid fever curve until chlorinization of the water supply was introduced in 1911, after which there was a distinct drop. As has been pointed out, the water supply was never obtained from a single source until 1915. The advantages which should have accrued from the various changes in the water supply were never wholly reaped because the old conditions were but slowly replaced, the older and poorer supplies being never entirely abandoned. For instance, after the introduction of the Lake Roland supply, a large proportion of the population still clung to the old springs and wells, and after many of these were abandoned in the old City in consequence of the activities of the Health Department, new sections which derived their water supply entirely from springs and wells were taken into the City; then too, after the introduction of the Gunpowder water supply, the old Lake Roland water supply was still to a great degree mixed with it. There is reason to think that the Lake Roland water supply obtained from Jones' Falls had become seriously polluted before the greater number of wells and springs were closed and this water was substituted. While it probably prevented a marked increase in the death rate, it exerted no great influence on its actual reduction from its previous level. It would appear that an infected external was substituted for an infected internal water supply.

Sewerage.—It has been shown that until 1915, with the exception of a few private sewers leading by the streams into the Basin and Harbor, human excreta containing typhoid bacilli from the cases and carriers emitting them were deposited in or on the ground. Had all the water supply been extra-urban and had all the dejecta gone into the soil, amateur hygienists to the contrary notwithstanding, there could be no serious objections to this method on sanitary grounds. But the water supply, until a late date, was largely of intra-urban origin, and the City was very closely built up. However, the spaces intended for drainage in many if not most cesspools became stopped, and the latter in consequence took on the attributes of surface privies. At one time it was the fashion to build water-tight cesspools, and the contents of many of these overflowed into streets, alleys, courts, and cellars. Material from many of the cesspools and privies, therefore, reached the wells and springs from the surface as well as through the soil. This material from overflowing cesspools and privies, as well as from the night-soil dumps, scows, and carts, was readily accessible to flies, the breeding and feeding of which in swarms were favored by the conditions of the stables, the imperfect surface drainage, and the inefficient collection and disposal of garbage. The methods of disposal of human waste favored,

to a high degree, the spread of typhoid fever by water, flies, and by milk and other foods. With the development and the perfection of the extra-urban and the gradual abandonment of the intra-urban water supply, the cesspool and the surface privy lost much of their importance as sources of typhoid infection. It is evident, that if the wells and springs had been abandoned sooner, the privy and the cesspool, though still harborers, would early have lost in importance as distributors of typhoid infection. After 1890, infection from this source was practically confined to that conveyed by means of flies. It is clear, therefore, that so far as concerns action other than from sources of infection for intra-urban drinking water, and the milk, and perhaps other foods, the sewerage remained a rather constant and relatively unimportant factor in the spread of typhoid until the new system was brought into general use in 1915. After 1915, it was possible for the sewerage system to exert in this direction but comparatively little influence on typhoid fever.

Milk.—During the whole period between 1851 and 1894, conditions were most favorable for the spread of typhoid fever by means of milk. In the first place, much of it was from cows kept within the City or nearby suburbs, where every chance existed for its pollution from actual cases of typhoid fever and from carriers. It was heavily watered, for much of the time with water from polluted wells and springs. It is not improbable that the substitution of less polluted water for watering milk before 1894 and the interference by the Health Department with watering after this date affected the typhoid death rate favorably. Under the laws of 1896 and 1902, the Health Department was able to improve very markedly the conditions under which milk was produced and sold within the City and thus to cut down very materially the opportunities for the dissemination of typhoid fever by milk produced and infected within the City. Under these laws, the urban cow was to a great extent but not entirely driven out. While these laws did not forbid the keeping of dairy cows within the City, they made it impossible to keep them within the closely built up sections and, on the whole, unprofitable, on account of the expense of constructing stables of the type required to keep them at all except in certain outlying districts. A great revolution in the control of the milk supply followed the enforcement of the milk ordinance of 1908, and by 1912 considerable oversight and control had been gained by the Health Department over the milk supply on dairy farms as well as within the City itself. Still between 1912 and 1918, numerous larger and smaller milk outbreaks were traced by the Health Department. As late as 1917, nearly 8 per cent of the reported cases were traced to milk in readily recognizable outbreaks. Besides these, there must have been large numbers of isolated or small groups of cases, in which the infection was carried by milk and which the machinery of the Department was inadequate to detect. The regulations of the Health Department could not, of course, prevent the removal of empty milk bottles from households with typhoid fever patients until such cases had been reported, and under the most favorable conditions two or three weeks pass between the beginning of the illness and the report of the case. Unless milk bottles are uniformly sterilized as a matter of routine, bottled milk may be, during the prevalence of typhoid, more dangerous than milk served from the old-fashioned churn. When milk is sold from the churn or can, the purchaser receives it in his own container, and the dairy takes nothing infected away from the householder. The compulsory sale of milk in bottles without adequate provision for the cleaning and sterilization of the latter is a sanitary reform of doubtful value from certain standpoints. Until 1918, the refrigeration of the milk supply, whether in churns, cans, or bottles, was, with certain notable exceptions, quite inadequate, and in warm weather conditions were favorable for the multiplication of typhoid bacilli that reached milk.

It is only since the enforcement of the milk ordinance of 1917 that there has been reasonable security against milk-borne (including ice cream) typhoid in Baltimore. This law requires not only pasteurization and refrigeration of the general milk supply, but the cleansing and sterilization of all milk utensils, including the farmers' cans. No cases of typhoid fever were traced to milk in 1918 and 1919. It must be borne in mind, however, that owing to the great diminution in the number of cases of the disease in rural Maryland, as well as within the City, the opportunities for the infection of milk have become correspondingly less.

Importation of Cases and Carriers: (a) Immigrants.—It is well established by the writings of Nathan Smith and Bartlett, that typhoid fever was prevalent in New England before 1826 and was well established there as the most common continued fever by 1842. Some years later, Bartlett showed that the disease was widely diffused throughout this country, south as well as west.

From 1800 to 1850, Baltimore grew very rapidly by immigration; the chief sources of immigration from abroad being England, Scotland, Ireland, and Germany. There was during this time, especially in the earlier years, a large immigration from New England and Pennsylvania, as well as from Virginia. In all of these places typhoid fever was then widely prevalent, and it is therefore certain that these immigrants as clinical cases and carriers contributed largely to the spread of the disease, doubtless long before strongly entrenched here. The later European immigrations were all from typhoid stricken countries. Baltimore's great prominence as a port city added to this danger. In the lower part of the City, where recent immigrants mostly settled or lodged, while transients, conditions were especially favorable for contamination of the wells and springs and for the spread of the infective agent by contact and by flies.

Importation of Cases and Carriers: (b) Military.—For the ten-year period before the Civil War, the average mortality rate for typhoid fever in Baltimore was about 45 per 100,000. From 47.09 in 1860 it jumped to 75.92 in 1861, remaining high during the whole war period and for some years afterwards. The highest portion of the typhoid mortality curve lies between 1861 and 1872. During the whole war, Baltimore and its vicinity were crowded with federal troops brought

from widely separated parts of the country and from the hospitals and battle-fields of Virginia, West Virginia, and probably Kentucky, and perhaps further south and west. The City became a great collecting and distributing point for troops. There were a number of military hospitals, some very large, for the treatment not only of the wounded, but of the sick. All the facts favor the inference that the presence in the City of great numbers of transient soldiers of an army known to be highly affected with typhoid fever must have resulted in an increase in the morbidity and mortality rates of this disease among the inhabitants. It is very probable that the soldiers were an important factor in spreading infection, not only through the wells and springs within the City, but over the Lake Roland watershed.

Importation of Cases and Carriers: (c) Isolated Individuals.—In addition to the large immigrations, attention should be called to the fact that large numbers of cases were annually brought into the City for hospital treatment, and even of late years a not inconsiderable proportion of the typhoid fever cases and deaths have occurred among individuals, from other states and on foreign ships, who have arrived in the City with the disease.

It has been pointed out above that for the three years just past, 20 per cent of the total deaths of typhoid fever in Baltimore occurred among individuals brought in to Baltimore hospitals from rural Maryland for treatment. On the whole, it seems probable that the mortality rate for typhoid fever in Baltimore has been, throughout the history of this disease, materially influenced by the importation of isolated cases of the disease.

Infection Contracted Without the City.—As far back as 1890, it was recognized in the Health Department that a considerable proportion of the cases of typhoid fever reported in Baltimore was contracted without the city limits, either in rural Maryland or in other states. It has been shown that in 1919 over 12 per cent of the reported cases certainly received their infection outside of the City and that 8 per cent probably had this source of origin. Until the last few years, there is reason to think that many cases were infected at resorts on the Bay and the numerous small rivers and estuaries of the Bay, near the City, particularly at the so called "shores." When the great prevalence of typhoid fever, until recent years, in rural districts of Maryland and other states, which Baltimoreans visited for work or pleasure, is taken into consideration, it seems probable that in the past this source of infection was even more important than at present.

In this connection, it is significant that the morbidity rate for Baltimore has declined in parallel with that of rural Maryland, particularly in the counties adjacent to Baltimore, in which the greater proportion of infection took place, and that nearly all of the cases of typhoid fever, attributed to these sources of infection within the last few years, have occurred in the warm weather when typhoid fever is most prevalent in the country districts.

Associative Contact with Recent Cases and with Remote Cases; i. e., Carriers.—If 12½ per cent of the cases could be traced to contact with recent cases in households and hospitals and nearly 8 per cent to contact with remote cases in households, *i. e., carriers,* in 1918 and 1919, it is reasonable to suppose that these factors exerted at least as great an influence upon the people in former years, since their conditions of life and their habits were much more favorable for infection by associative contact than at present.

Flies.—Enough has been said under other headings to indicate that until very recently the general sanitary conditions of the City were peculiarly adapted to fly-borne typhoid. It remains to be pointed out that, particularly in the crowded sections of the City and among foreigners, the great numbers of flies had ready access to the excreta of typhoid patients. The fly season is a long one, screening was almost unknown, and among certain elements in the population, the screening of the apartments of typhoid fever patients was enforced with great difficulty or not at all.

Bathing in Polluted Waters.—It is probable that until the last few years, this has been a frequent source of infection of typhoid fever within the city limits. It is not improbable that many persons were infected by bathing in the polluted streams within the city limits, as well as in the Basin and in the middle branch of the Patapsco at Spring Gardens.

UNEXPLAINED CASES

The question of the source of infection of the group of *unexplained cases* must be faced. Even if it be granted that the explanations offered for the *explained cases* are adequate—and the value of some of these (probable and possible out-of-town infections, infection from remote cases in which carriers were not demonstrated by culture methods, and neighborhood infection by flies) is acknowledged to be questionable—there still remains a large group of cases upon the origin of which no light has been thrown.

In the absence of some other obvious explanations, it is natural and usual to attribute these cases to water and milk-borne infection. But slight consideration shows this to be unsatisfactory, and the same question arises in all cities with comparatively low and falling typhoid fever rates, following the inauguration of apparently effective water supply and sewerage and milk protection systems. In the old twenty-four wards of Baltimore, the sewerage system—with the exception of certain districts which, however, do not show any particular difference in typhoid incidence—may be elminated from consideration. If any dependence can be placed upon the value of the bacteriological examinations conducted independently in the laboratories of the filtration plant and of the Health Department during 1919, the city water supply in that year at least, according to the accepted and apparently logical standards, may be acquitted. The number of reported cases on the watershed were very few and were supervised both by the Water Department and by the very efficient deputy State Health Officer. If the water supply were responsible for some or even most of the cases now under consideration, it is reasonable to suppose that there should have been an even greater number than actually occurred.

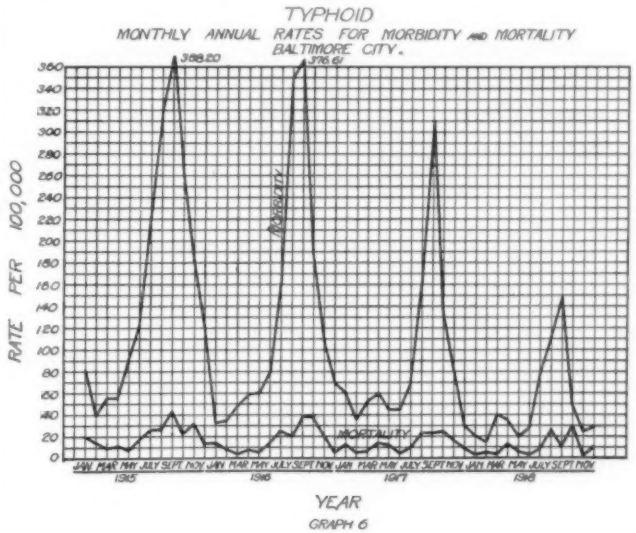
To state that no cases were traced to milk is not equivalent to asserting that infection was not spread through this medium. It is unwarrantable to assume that all chances of the spread of the disease by milk were successfully guarded against. No one concerned with the oversight of the milk supply thinks that all the milk utensils were sterilized and that all the milk was adequately pasteurized. There is still a chance that some typhoid fever in Baltimore is spread by milk. However, if the milk supply was even an important source of infection for this group of cases, explanation would still be lacking for the comparatively large proportion of these cases in which the use of milk as a beverage was denied.

When this *unexplained* group is analyzed, it is found that the cases do not vary essentially from the *explained* group in age, sex, color, or seasonal and locality distribution. They vary in only one attribute.

It is certain that, in some cases at least, more complete histories could have been elicited. Some of the patients were not only illiterate and stupid, but many could not express themselves in languages familiar to the investigators. It must also be recalled that many of the patients were too ill at the time of the investigations to give clear answers and to recall all the details of their histories. In such cases, the investigators had to rely upon the testimony of relatives and friends. Again, it is not possible for an individual to recall all his actions and contacts some weeks after an event. After all, the modes of transmission inquired into and which we have been considering are only the very obvious and perhaps there are others quite as important.

There are in this community hundreds of survivors of clinically recognized and unrecognized attacks of typhoid fever who are potentially carriers and who from time to time may pass on the infective agent to others. A colored cook, proven to be a carrier responsible for 9 cases of typhoid fever among college girls and who appeared to be quite frank when submitted to the closest interrogation, was unable to recall a febrile illness suggestive of ordinary clinical typhoid fever. She described, however, very clearly the symptoms of an attack of acute cholecystitis which she had had about a year before. Her story was confirmed later, even as to dates, by her physician. She apparently had had an attack of primary cholecystitis due to *B. typhosus* and resulting in the carrier state. Had she not by chance transferred typhoid infection to a number of college girls at the same time, she would probably not have been apprehended. Upon such chances as these the transmission of this disease and the recognition of its mode often depend. None of this woman's personal associates gave a history of frank typhoid fever either before or after her attack of cholecystitis. The victim of typhoid fever rarely knows the typhoid fever history of his intimate associates outside of his family, and, when questioned, cannot tell whether any of his hosts or hostesses or their servants have had the disease. To find a hidden carrier, it would be necessary to take a census of the patient's known and unknown contacts. A large proportion of the several hundred food handlers whom I have had cultured with negative results may be intermittent carriers, infecting from time to time people not intimately associated with them. There must be in this community a considerable number of so called "healthy carriers," typhoid carriers who have not developed clinical typhoid fever and who are effective infectors, or who may become self-infected; *i. e.*, their barriers of resistance may at some time become ineffective against typhoid bacilli on the surface of their bodies (on the mucosæ of the biliary, intestinal, or urinary tracts). It is impossible to set a time limit between the beginning of the healthy carrier state and true infection.

If butter and certain kinds of cheese were exhaustively studied, with more refined methods than now exist for isolating *B. typhosus*, it is probable that it would be found that



GRAPH 6

these foods are a not uncommon means for the transmission of typhoid fever in urban communities.

Now that the City Fathers, after many years of fumbling, have finally made those general sanitary provisions necessary for the reduction of the incidence of typhoid fever to a comparatively low level, in the old twenty-four wards, and the obvious may be set to one side, the public health administrator of an inquiring turn of mind may turn himself to the much more interesting and important problem of determining the less obvious or hidden means of transmission of the disease.

SEASONAL DISTRIBUTION

It has long been held that typhoid fever in Baltimore is essentially a disease of the summer and autumn. This has not always been the case, for during many years, from 1856 to 1881, the number of deaths in the winter months—December, January, and February—equalled or even exceeded those for the summer and fall months. It is interesting that this was the period during which so much of the water supply was intra-urban in origin and when the extra-urban supply was entirely or mainly derived from Jones' Falls. Since 1881, the highest monthly mortality has remained permanently in the summer and fall months. During this period, the springs

and wells in the City as a whole were abandoned and the Gunpowder water supply was largely or entirely substituted. As long as the water supply was grossly and steadily polluted, the distribution of the disease tended to be more evenly distributed throughout the year.

A study of the reported cases since 1900 shows that from February, the month of lowest incidence, there is a very gradual progression of the disease until July when there is a rapid rise, culminating in a peak usually about the middle of September and more rarely late in August, or even early in October. The decline is usually somewhat abrupt during September or October and more gradual in November, December, and January. The deaths follow the same general course, lagging about one month behind the reported cases. This course of the disease is well shown on Graph 6, on which the monthly annual morbidity and mortality rates are given for the years 1915 to 1918, inclusive.

Analysis of the modes of action of the factors influencing the incidence of typhoid fever in Baltimore makes clear why, during the second period, the greatest incidence and mortality should be in the warm months. This is the season when the greatest number of imported cases occur, when conditions are most favorable for infection by means of milk, milk products, and other foods (and in which consequently most of the observed milk epidemics have occurred), when there are the greatest number of cases upon the watersheds, and when conditions are auspicious for the dissemination of the infective agent by carriers, clinical cases, flies, and bathing in polluted waters.

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